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**Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.**

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3 **Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a**  
4 **randomized controlled trial to determine feasibility and safety of using**  
5 **increased dialysate magnesium concentrations to increase plasma**  
6 **magnesium concentrations in people treated with hemodialysis.**  
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42 **Abbreviations**

43 AE, adverse event; CKD, chronic kidney disease; CRP, C-reactive protein; ECG,  
44 electrocardiography; PAC, premature atrial complex; PWV, pulse wave velocity; PTH,  
45 parathyroid hormone; PVC, premature ventricular complex; SOC, standard of care; SAE,  
46 serious adverse event; SPIRIT, Standard Protocol Items: Recommendation for Interventional  
47 Trials (SPIRIT)  
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## **Abstract**

### ***Introduction***

People treated with hemodialysis are at increased risk for all-cause and cardiovascular mortality. Plasma magnesium concentration has been inversely associated with these risks. Therefore, plasma magnesium may be a new modifiable risk factor and an increase of dialysate magnesium concentration may be an easy, safe and effective way to increase plasma magnesium concentrations. Detailed information on modulating dialysate magnesium concentrations is limited in literature. Primary objective of this study is to determine the safety and feasibility to increase plasma magnesium concentrations in people treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate.

### ***Methods and analysis***

In this randomized double blinded standard of care controlled trial, fifty-three persons treated with hemodialysis, will be randomly allocated 2:1 to either a step-wise individually titrated increase of dialysate magnesium concentration from 0.50 to 0.75 to 1.00 mmol/L during 8 weeks, or a standard dialysate magnesium concentration of 0.50 mmol/L. Other study measurements include dietary records, questionnaires, electrocardiography (ECG), Holter registration and pulse wave velocity. The primary endpoint is pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8. In addition, the predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored using linear mixed models. Safety endpoint is defined by the occurrence of hypermagnesemia above 1.25 mmol/L, or bradycardia or prolonged QTc interval detected on the ECG.

### ***Ethics and dissemination***

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center. The results of the study will be disseminated by publication in peer-reviewed scientific journals and presentation at national or international conferences in the field of interest.

### ***Trial registration number***

NTR 6568 / NL6393

### Strengths and limitations

- Blinding and randomization prevents bias occurring from differences in life-style between groups, and enables objective collection and processing of data
- Dialysate concentrations will be individually titrated based on individual plasma magnesium concentrations by an independent physician
- Not only the effects of dialysate magnesium concentrations on plasma magnesium concentrations will be determined, but also the factors that are predictive for these effects
- Major limitation is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to limited study duration

## Introduction

People with chronic kidney disease (CKD) including those treated with dialysis are at increased risk for all-cause and cardiovascular mortality.<sup>1</sup> This increased risk persists after adjustment for traditional cardiovascular risk factors, indicating that other kidney specific factors contribute to the cardiovascular risk.<sup>1</sup> Recently, lower magnesium has been identified as a potential novel risk factor.<sup>2</sup> Magnesium is involved in many physiological functions, including energy metabolism and regulation of transmembrane transport of ions and consequently, it is essential for muscle function, cardiac rhythm and vascular tone.<sup>3</sup> In a meta-analysis of studies in people with CKD including those on dialysis, we showed that plasma magnesium concentration is inversely associated with all-cause and cardiovascular mortality, and that this not only applies for normal compared to low magnesium, but also revealed protective effects of magnesium above as compared to within the reference range (generally 0.70 – 1.05 mmol/L).<sup>4</sup> Magnesium concentration is also inversely associated with the risk for sudden death and with arrhythmia in people treated with hemodialysis.<sup>5-7</sup> In the general population, serum magnesium has been inversely associated with frequent or complex premature complexes, which predict prognosis including all-cause mortality in the general population.<sup>8,9</sup> Moreover, serum magnesium was inversely associated with pulse wave velocity (PWV) in people treated with maintenance hemodialysis,<sup>10</sup> and in a short randomized cross-over study in people treated with hemodialysis, higher dialysate magnesium concentration compared to standard dialysate magnesium concentration decreased pulse wave velocity.<sup>11</sup> PWV is a marker of vascular stiffness and a strong predictor of cardiovascular outcome in people with CKD stage 4-5D.<sup>12</sup> In most studies that included multiple categories of plasma magnesium concentration in people with CKD including those treated with dialysis, there was a monotonic inverse association between magnesium and all-cause mortality.<sup>4</sup> We previously showed that a commonly used dialysate magnesium concentration of 0.50 mmol/L in hemodialysis, usually induces a decline of magnesium towards magnesium concentrations in the lower range of normal.<sup>13</sup> Therefore, an increase of dialysate magnesium concentration, may be an easy, safe and effective way to increase plasma magnesium concentrations in people treated with hemodialysis, without the need of oral supplementation. The results of one observational study suggest that there may be an optimal concentration of plasma magnesium in-between 1.15-1.27 mmol/L, with an increasing risk for mortality if magnesium values exceed this range.<sup>14</sup> Although these

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3 findings were not confirmed by other studies that included magnesium values in this high  
4 range, this requires to take into account safety when increasing plasma magnesium  
5 concentrations.<sup>6, 15, 16</sup> In a previous 4-weeks trial by Bressendorf et al., increasing dialysate  
6 magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase of  
7 plasma magnesium concentration (95%-CI 0.3-0.5).<sup>17</sup> Here, we describe a randomized  
8 standard-of-care controlled trial of step-wise increment of dialysate magnesium  
9 concentration in people treated with hemodialysis. Primary objective is to determine the  
10 feasibility to increase plasma magnesium concentrations in individuals treated with  
11 hemodialysis by means of sequentially increasing concentration of magnesium in the  
12 dialysate. Secondary objectives are to determine safety of using higher dialysate magnesium  
13 concentrations, the effect of dialysate magnesium on plasma magnesium concentration,  
14 and to define which parameters are predictive for the increment of plasma magnesium  
15 concentration by increasing dialysate magnesium. We will also explore the effects of using  
16 higher dialysate magnesium on cardiac rhythm and pulse wave velocity.  
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## 33 **Methods**

### 34 ***Trial design***

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36 In this randomized, double-blind, standard of care (SOC) controlled multi-center trial,  
37 individuals treated with hemodialysis will be randomly allocated to either a stepwise  
38 increase of dialysate magnesium concentration from 0.50 to 1.00 mmol/L, or continue on a  
39 standard dialysate magnesium concentration of 0.50 mmol/L. The protocol was written in  
40 accordance with the Standard Protocol Items: Recommendation for Interventional Trials  
41 (SPIRIT) and registered at [www.trialregister.nl](http://www.trialregister.nl) (registration number NTR 6568 / NL6393).<sup>18</sup>  
42  
43 The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and  
44 was approved by the Ethical Committee of the VU University Medical Center (registration  
45 number 2017.408, NL62679.029.17).  
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### 54 ***Characteristics of participants and recruitment***

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56 Adult persons treated with hemodialysis on a 3-times weekly dialysis schedule, will be  
57 enrolled in the study. The in- and exclusion criteria are listed in table 1. Participants will be  
58 recruited from multiple centers in the Netherlands, including Amsterdam University Medical  
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Center location VU Medical Center, Amsterdam; Diapriva dialysis center, Amsterdam; Niercentrum aan de Amstel, Amstelveen; and Spaarne Gasthuis, Hoofddorp. Participants need to provide written informed consent prior to enrollment.

**Table 1.** Inclusion and exclusion criteria

Inclusion criteria
<ul style="list-style-type: none"> <li>• Age <math>\geq 18</math> years</li> <li>• Hemodialysis with regular three times weekly dialysis schedule</li> <li>• Hemodialysis since at least 3 months</li> <li>• Standard dialysate magnesium concentration 0.50 mmol/L</li> <li>• Providing informed consent</li> <li>• Pre-dialysis plasma magnesium concentration <math>\leq 1.00</math> mmol/L after the long intra-dialytic interval</li> </ul>
Exclusion criteria
<ul style="list-style-type: none"> <li>• Intravenous magnesium supplementation (including total parenteral nutrition) in the last 2 weeks</li> <li>• Expected cessation of dialysis treatment within three months after inclusion or expected permanent or temporary dialysis center switch to a center not participating in the trial within three months after inclusion.</li> <li>• Prolongation of QTc interval: male <math>&gt;450</math> ms or female <math>&gt;460</math> ms on baseline ECG</li> <li>• Bradycardia: heart rate below 60 beats per minute on baseline ECG</li> <li>• Chronic arrhythmia or cardiac conduction disorder other than atrial fibrillation or ventricular extrasystole that poses the patient at risk at the discretion of the treating physician.</li> <li>• Change of proton pump inhibitor prescription in the last 2 weeks</li> </ul>

*In order to be eligible to participate in this study, a subject must meet all of the inclusion criteria. A potential subject who meets any of the exclusion criteria will be excluded from participation in this study.*

### **Intervention**

In the intervention group, dialysate magnesium is increased stepwise, from 0.50 mmol/L at baseline, to 0.75 mmol/L during week 1-4, and to 1.00 mmol/L during week 5-8. The participant proceeds to the next increment step of dialysate magnesium concentration after week 4 only if pre-dialysis plasma magnesium after the long interdialytic interval is below 1.15 mmol/L in week 4. Otherwise, the dialysate magnesium concentration of 0.75 mmol/L is continued in week 5-8. After week 8, dialysate magnesium will be gradually reduced with 0.25 mmol/L in week 9 and thereafter return to the standard dialysate magnesium concentration of 0.50 mmol/L in week 10.

Participants in the control group are treated with a standard dialysate magnesium concentration of 0.50 mmol/L. (see Figure 1)

The dialysate contains a potassium concentration of 2 or 3 mmol/L, as determined in routine care by the treating physician based on individual needs. For the respective



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3 magnesium concentrations, six dialysis concentrates are used in weeks 1-9 (Hemodialysis A-  
4 concentrate, D761, D987, D907, D283, D961 and D908, MTN Neubrandenburg GmbH,  
5 Neubrandenburg, Germany). In the composition of these concentrates, besides potassium  
6 based on individual needs, only the amount of magnesium chloride is different.  
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### 10 11 12 **Study procedures and participant time line** 13

14 The study procedures and participant time line are shown in Figure 2. After informed  
15 consent is provided by participants that meet in- and exclusion criteria, blood sampling and  
16 electrocardiography (ECG) are performed at baseline. Eligible persons meeting the criteria  
17 for plasma magnesium and ECG, as provided in table 1, are allocated to the either SOC or  
18 incremental magnesium dialysate. During the trial, blood sampling will be performed before  
19 and after the dialysis sessions following the long interdialytic interval weekly, and every  
20 dialysis session in week 1 and 5 to measure plasma magnesium concentration. In addition,  
21 in week 1, 5 and 9, blood is collected for measurements of potassium, bicarbonate, calcium,  
22 albumin, phosphate, parathyroid hormone (PTH), hemoglobin and C-reactive protein (CRP).  
23 For laboratory measurements, standard methods of the local laboratory are used.  
24 Participants record dietary intake for 3-days at baseline. From this record, dietary  
25 magnesium intake is extracted using the Dutch Food Composition Database (NEVO) by using  
26 the calculator on the website of the Dutch Nutrition Center.<sup>19, 20</sup> A questionnaire regarding  
27 the presence of subjective symptoms that can be associated with hypermagnesemia is  
28 completed at baseline, week 4 and 8. This is a 7-point yes or no questionnaire to ask if a  
29 participant experienced the following symptoms in the last week: nausea, vomiting,  
30 dizziness, drowsiness, reduced muscle strength, itching and leg cramps, and at baseline, also  
31 a question about chronic diarrhea and over-the-counter use of magnesium supplements is  
32 included. An electrocardiogram (ECG) is repeated before dialysis in week 4 and 8 to  
33 determine heart rhythm, frequency and QTc interval. In addition, at baseline and in week 8,  
34 participants undergo continuous heart rhythm monitoring and pulse wave velocity (PWV)  
35 measurements. Heart rhythm monitoring is performed using a Holter recorder (Fysiologic,  
36 Amsterdam, The Netherlands) for 48 hours including one dialysis sessions and one  
37 interdialytic period. Carotid-femoral PWV is measured using the Sphygmocor tonometer  
38 (Atcor Medical Pty Ltd, Software version 9.0, Sydney, Australia) preceding the midweek  
39 dialysis sessions. Participants are requested to avoid coffee, tea and smoking for 4 hours,  
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3 and alcohol for 12 hours preceding the measurements, as is recommended by the  
4 manufacturer of the device. The patient is placed in supine position, in a quiet environment  
5 at room temperature. After attachment of ECG electrodes and bedrest for at least 5  
6 minutes, blood pressure is measured with an automated Omron device at least 3 times with  
7 a few minutes in-between, and until no substantial change occurs. Then, the last blood  
8 pressure measurement is recorded. The carotid to femoral artery distance is measured  
9 directly and multiplied by 0.8 to estimate the difference between cardiac-carotid and  
10 cardiac-femoral distance as recommended by expert consensus.<sup>21</sup> The pulse wave is  
11 recorded using the tonometer at the carotid and femoral site and then PWV is calculated  
12 automatically by the Sphygmocor software from entered blood pressure, distance and ECG  
13 and tonometer recordings. Measurements are performed at least twice or until two  
14 measurements are of appropriate quality. A measurement is considered of sufficient quality  
15 based on the criteria set by the manufacturer: adequate shape of detected signal of ECG  
16 and pulse wave, difference of heart rate  $\leq 5$  bpm between carotid and femoral  
17 measurement, ECG R-tops and pulse wave beginning are correctly identified by the  
18 software, and standard deviations of the ECG to carotid and of ECG to femoral time are both  
19  $< 6\%$ . PWV measurement is not performed in participants with irregular heart rhythm,  
20 pacemaker rhythm, atrial fibrillation or flutter, heart frequency below 40 or above 160 bpm,  
21 2<sup>nd</sup> or 3<sup>rd</sup> degree atrioventricular block, severe aortic valve stenosis or instable carotid  
22 plaque, as contra-indicated by the manufacturer. Furthermore, persons' characteristics are  
23 recorded at baseline and characteristics of the dialysis are recorded weekly for the time of  
24 dialysis after the long interdialytic interval and for every dialysis during the first and fifth  
25 week of intervention. Medication use and dosage is recorded at baseline and at week 8.  
26 During the trial, all participants receive three times weekly hemodialysis sessions according  
27 to their regular schedule. Changes of dialysis schedule during the study will be avoided as  
28 much as possible if there is no medical indication to change the scheme. Also, changes in  
29 prescription of proton pump inhibitors and magnesium-containing supplements, laxatives  
30 and phosphate binders will be avoided if clinically allowed.

### 56 ***Safety monitoring***

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58 If participants in the intervention group reach a plasma magnesium of 1.25 mmol/L or  
59 above, as noted by an unblinded independent nephrologist not involved in the trial (see  
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3 below), at any time point during follow-up in week 1 to 8, dialysate magnesium  
4 concentration will be reduced to the previous level in the next week. If plasma magnesium  
5 remains 1.25 mmol/L or above in the next week in intervention phase 2 (week 5-8),  
6 dialysate magnesium is further reduced one step (to 0.50 mmol/L). If a participant develops  
7 bradycardia with heart rate below 50 beats per minute (bpm), or prolonged QTc interval  
8 (>450 ms in male or >460 ms in female), noticed on electrocardiography (ECG) in week 4,  
9 dialysate magnesium concentration is also reduced to the previous step. (Figure 1) Plasma  
10 magnesium above 1.25 mmol/L, bradycardia < 50 bpm and prolonged QTc will be recorded  
11 as adverse event (AE). Serious adverse events (SAE's) will be recorded and reported to the  
12 primary investigator and medical ethical committee.  
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### 23 **Main endpoints**

24 Primary endpoint is pre-dialysis plasma magnesium concentration after the long  
25 interdialytic interval at the end of week 8, in the intervention group compared to the  
26 control group. The change of pre-dialysis plasma magnesium concentration after the long  
27 interdialytic interval from baseline to the end of week 8, will also be determined in the  
28 intervention group compared to the control group.  
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### 36 **Secondary endpoints**

37 Post-dialysis plasma magnesium concentrations after the dialysis sessions following the long  
38 interdialytic interval will also be determined. Safety endpoint is the safety of using higher  
39 magnesium concentrations in the dialysate, as indicated by the incidence of respectively  
40 hypermagnesemia (>1.25 mmol/L) at any timepoint, or bradycardia (defined as heart rate  
41 below 60 bpm) or prolonged duration of QTc interval (>450ms in male or >460ms in female)  
42 identified on the ECG in week 4 or 8. Other explorative endpoints include the change of  
43 PWV from baseline to week 8; and the number of complex premature ventricular complexes  
44 (complex PVC's), premature atrial complexes (PAC's), and heart rate variability as detected  
45 with Holter ECG monitoring. Complex PVC's are defined as PVC's that are multiform,  
46 repetitive or have a frequency of >30/h.<sup>8</sup> Furthermore, the following outcomes will be  
47 recorded: subjective symptoms that can be associated with hypermagnesemia determined  
48 from self-reporting in questionnaires in week 4 and 8; hospitalization; mortality; and  
49 cardiovascular events that lead to hospitalization or mortality including arrhythmia, cardiac  
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3 arrest, acute coronary syndrome, cerebrovascular accident, and hemorrhage from ruptured  
4 aneurysms of the abdominal aorta.  
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### 8 ***Blinding and randomization***

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10 Participants that fulfill all screening criteria, will be randomly allocated 2:1 in tranches of 6  
11 to either the intervention group or the control group by the pharmacy according to a  
12 computer-generated random list. Participants, treating physicians, nurses and researchers  
13 are blinded to treatment allocation. Dialysate cannisters are re-labelled by the pharmacy for  
14 the individual participant per individual study week. Labels include information on  
15 participant name, date of birth, study week and dialysate potassium concentration, but no  
16 further information on dialysate composition. For the first study week, the pharmacy  
17 chooses the appropriate dialysate concentrate based on treatment allocation and the in  
18 routine individual care determined potassium concentration. From week 2 on, one  
19 independent nephrologist that is not blinded for treatment allocation, weekly decides upon  
20 the dialysate magnesium concentration, after review of plasma magnesium concentrations  
21 according to the algorithm shown in Figure 1. The pharmacy then re-labels the dialysate as  
22 prescribed by the independent nephrologist.  
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### 36 ***Sample size calculation***

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38 We previously performed a study to determine plasma magnesium concentrations and  
39 variability in people receiving 3-times weekly hemodialysis treatment with a standard  
40 dialysate magnesium concentration of 0.50 mmol/L.<sup>13</sup> That study showed a mean pre-  
41 dialysis plasma magnesium concentration of  $0.88 \pm 0.14$  mmol/L.<sup>13</sup> After excluding pre-  
42 dialysis plasma magnesium levels above 1.00 mmol/L from the analysis, mean pre-dialysis plasma  
43 magnesium level was 0.83 mmol/L in that study population. Based on these results, we  
44 expect a mean plasma magnesium concentration of  $0.83 \pm 0.14$  mmol/L in the control  
45 group. Based on the results from the CONTRAST cohort analysis, in which plasma  
46 magnesium was associated with all-cause and cardiovascular mortality, we consider an  
47 increase of plasma magnesium concentration to 0.96 mmol/L in the intervention group  
48 relevant, which is a 0.13 mmol/L rise.<sup>5</sup> The required sample size calculated for two  
49 independent groups, based on the values just mentioned, a power of 0.80, probability of  
50 0.05, and 2:1 randomization, would be 28 in the intervention and 14 in the control group. To  
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3 account for an estimated drop-out of 20%, the required sample size is 53 participants in  
4 total: 35 in the intervention and 18 in the control group.  
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### 8 ***Statistical analysis***

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10 Continuous variables will be expressed as mean and standard deviation (SD) for normally  
11 distributed variables or median and interquartile range (Q1-Q3) for non-parametric  
12 distributed variables. Categorical variables will be presented as number and percentage. The  
13 primary endpoint, pre-dialysis plasma magnesium after the long interdialytic interval at the  
14 end of week 8, will be compared between the intervention and control group, using  
15 univariable analysis with an unpaired Student's t-test if variables are normally distributed (if  
16 necessary after logarithmic transformation) and with Mann-Whitney U test if variables are  
17 non-normally distributed. The analysis is performed as intention to treat, including all  
18 participants that are still in study follow-up at week 8. The change of plasma magnesium  
19 from baseline to week 8, is first analyzed within each group using a paired Student's t-test,  
20 and then the difference from baseline to week 8 (delta) is compared between the groups  
21 using linear mixed models. The predictive effect of dialysate magnesium concentration and  
22 other baseline parameters and dialysis characteristics on plasma magnesium concentration,  
23 will be explored in two separate analyses (for pre-dialysis and post-dialysis concentration)  
24 using linear mixed models. In addition, we will explore which parameters are predictive for  
25 the increment of plasma magnesium concentration from baseline to week 8 after  
26 sequentially increasing dialysate magnesium concentration, using linear mixed models. For  
27 secondary endpoints, univariable analysis for within-group changes and between-group  
28 differences will be performed using respectively paired and unpaired Student's t-test or  
29 Mann-Whitney U tests for continuous variables, and chi-square or Fisher's exact test for  
30 dichotomous variables. Linear mixed models will be used for multivariable analysis of  
31 secondary endpoints with repeated measures.  
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### 52 ***Data management***

53 Data is recorded in electronic case report forms using the webbased software Castor EDC  
54 (Amsterdam, The Netherlands). All data is stored in coded form. The identification key is  
55 stored at the local study site only. Randomization codes are stored at the pharmacy. The  
56 randomization code will not be broken until follow-up of all participants is completed.  
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### ***Dissemination***

The results of this study will be offered for publication in international peer-reviewed journals. In addition, the results can be presented at national and international conferences and meetings in the field.

### ***Patient and public involvement***

This study protocol was reviewed and approved by the Dutch Kidney Foundation (DKF). A patient panel of the Dutch Renal Patients Association is involved in the review of research protocols submitted to the DKF. Investigators will communicate results to participants once the final results become available. The results will also be shared with patient organizations.

### **Discussion**

This study aims to determine feasibility, safety, and predictive parameters for the effect of using dialysate with higher magnesium concentration to increase plasma magnesium concentrations in people treated with hemodialysis. In addition, this study will explore effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

### ***Relation with previous studies***

In a previous study, we demonstrated that a commonly used dialysate magnesium concentration of 0.50 mmol/L generally induces a decline of plasma magnesium concentrations towards concentrations in the lower range of normal.<sup>13</sup> Detailed information in literature on the effects of increasing dialysate magnesium concentration on pre- and post-dialysis plasma magnesium concentrations and safety is sparse. Two other studies showed that a dialysate magnesium concentration of 0.75 mmol/L generally resulted in a relatively stable plasma magnesium concentration, with a mean pre-dialysis concentration of 1.2 mmol/L and mean post-dialysis concentrations of 1.1 up to 1.2 mmol/L.<sup>22, 23</sup> In another trial, a 4-weeks single-step increment of dialysate magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase (95%-CI 0.3-0.5) of pre-dialysis plasma magnesium concentration and a mean pre-dialysis plasma Mg concentration of  $1.4 \pm 0.2$  mmol/L.<sup>17</sup> That study did not perform ECG nor Holter monitoring. As outlined in the

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3 introduction, an observational study in people treated with hemodialysis found an inverse  
4 association between plasma magnesium concentrations and arrhythmia.<sup>8</sup> Moreover, in a  
5 short randomized cross-over study in people treated with hemodialysis, a dialysate  
6 magnesium concentration of 0.75 mmol/L compared to 0.50 mmol/L decreased pulse wave  
7 velocity.<sup>11</sup>  
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### 14 ***Strengths and limitations***

15  
16 The strengths of this study protocol are the blinding and randomization. Although the  
17 primary outcome is an objective outcome measure, blinding and randomization are  
18 essential to prevent bias occurring from changes in life styles including dietary magnesium  
19 intake. In addition, it is of relevance for objective collection and processing of data from  
20 questionnaires, pulse wave velocity measurements, electrocardiography, and reporting of  
21 SAE's. Another strength of this protocol is that dialysate magnesium concentration will be  
22 individually titrated based on individual plasma magnesium concentrations. In addition, not  
23 only the effect of dialysate magnesium will be determined, but also other factors that are  
24 predictive for this effect will be determined. The major limitation of this protocol, is that the  
25 study will not provide information on clinical outcomes including cardiovascular events and  
26 mortality, due to a limited duration of the study.  
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### 38 ***Potential impact***

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40 The study described in this protocol may provide relevant information on the effect of  
41 dialysate magnesium on plasma magnesium concentrations, the strategy for titration of  
42 dialysate magnesium based on individual needs, the safety of increasing plasma magnesium  
43 concentrations, and about factors that are predictive for the effect of dialysate magnesium  
44 on plasma magnesium concentration. This information may enable to safely increase plasma  
45 magnesium concentrations by individualized dialysate magnesium concentrations. In  
46 addition, this study will provide explorative data about the effects of increased dialysate  
47 magnesium concentration on intermediate cardiovascular outcomes including cardiac  
48 rhythm and PWV. The information provided by this study, may pave the way to larger long-  
49 term randomized controlled trials on the effects of increasing plasma magnesium  
50 concentrations on clinical outcomes including all-cause and cardiovascular mortality in  
51 people treated with hemodialysis. If plasma magnesium indeed improves clinically relevant  
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3 outcomes, and can be safely increased by means of individualized increasing dialysate  
4 magnesium concentrations, potentially large health benefits may be achieved if magnesium  
5 is increased slightly above the reference range by an increase of dialysate magnesium  
6 concentration. If so, the cost-effect ratio is likely low, as raising magnesium concentration is  
7 an inexpensive intervention. In addition, it would be an easy intervention that needs no  
8 additional patient effort and no oral supplementation would be needed in these persons  
9 that already experience a high pill burden. Therefore, the study described in this protocol  
10 may provide information of high relevance to patients, clinicians and health care providers  
11 and may eventually help to decrease morbidity and mortality in people treated with  
12 hemodialysis.  
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### 23 ***Safety considerations***

24 Plasma magnesium concentrations are expected to rise up to values above the reference  
25 range by the increase of dialysate magnesium concentration. However, clinical symptoms of  
26 hypermagnesemia, typically are not observed before plasma magnesium concentrations  
27 exceed 2.0 mmol/L, which is high above the target concentrations in this study. Reported  
28 symptoms of hypermagnesemia (if plasma concentrations are above 2.0 mmol/L) include  
29 lethargy, drowsiness, flushing, nausea and vomiting, and diminished deep tendon  
30 reflexes.<sup>24</sup> In even more severe hypermagnesemia (plasma concentrations above 3.0  
31 mmol/L) also somnolence, loss of deep tendon reflexes, hypotension and ECG changes can  
32 occur, and in extreme hypermagnesemia (above 5.0 mmol/L) complete heart block, cardiac  
33 arrest, apnea, paralysis and coma have been reported<sup>24</sup>. As a result of hemodialysis inherent  
34 techniques, the increment of (free) plasma magnesium is restricted by its concentration in  
35 the dialysate (maximally 1.00 mmol/L in this study), so overshoot to symptomatic  
36 concentrations is virtually impossible. The risk for severe or symptomatic hypermagnesemia  
37 is further minimized by intensive monitoring. As dialysate magnesium concentration is not  
38 further increased after a plasma magnesium concentration of 1.15 mmol/L is reached, and  
39 is reduced if plasma concentrations of 1.25 mmol/L are reached at any time point, severe or  
40 symptomatic hypermagnesemia will be prevented. These maximal target concentrations are  
41 set taking into account an observational study in people treated with hemodialysis that  
42 suggested an optimal magnesium concentration in-between 1.15-1.27 mmol/L and  
43 increased risk for mortality if magnesium exceeds 1.27 mmol/L.<sup>14</sup> Furthermore, for safety  
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3 reasons, individuals with bradycardia or a prolonged QTc interval on the ECG at baseline will  
4 be excluded from participation, and in individuals with bradycardia with a heart rate below  
5 50 bpm or a prolonged QTc interval identified on the ECG in week 4, dialysate magnesium  
6 will be reduced. Based on these careful methods, the risk for individuals participating in this  
7 study is low. Considering the limited burden and risks associated with this study and a  
8 possible highly-relevant contribution to future improvement of treatment and prognosis in  
9 people treated with hemodialysis, the potential benefits outweigh the burden and possible  
10 risks.  
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### 20 **Trial status**

21 The trial is currently ongoing. The first participant was randomized on the 4th of April 2018  
22 and up till now 43 of 53 participants have been randomized.  
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### 26 **Funding**

27 This work was supported by the Dutch Kidney Foundation (PhD grant no. 15OP02) and the  
28 PPP Allowance made available by Top Sector Life Sciences & Health to The Dutch Kidney  
29 Foundation to stimulate public-private partnerships (grant no. LSHM17034-HSGF).  
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### 36 **Conflicts of interest statement**

37 MV received research grants from Vifor, Amgen, Fresenius, and acted as consultant for  
38 Medice, Astra Zeneca, Vifor, Amgen, Fresenius, Otsuma, Kyowa Kirin. The other authors  
39 declare no conflicts of interest.  
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### 46 **Authors contributions**

47 NL, MV and JH conceived the study. NL and MV designed the study. NL wrote the manuscript  
48 and MV and JH revised the manuscript. Each author approved the final version of the  
49 manuscript.  
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### 55 **References**

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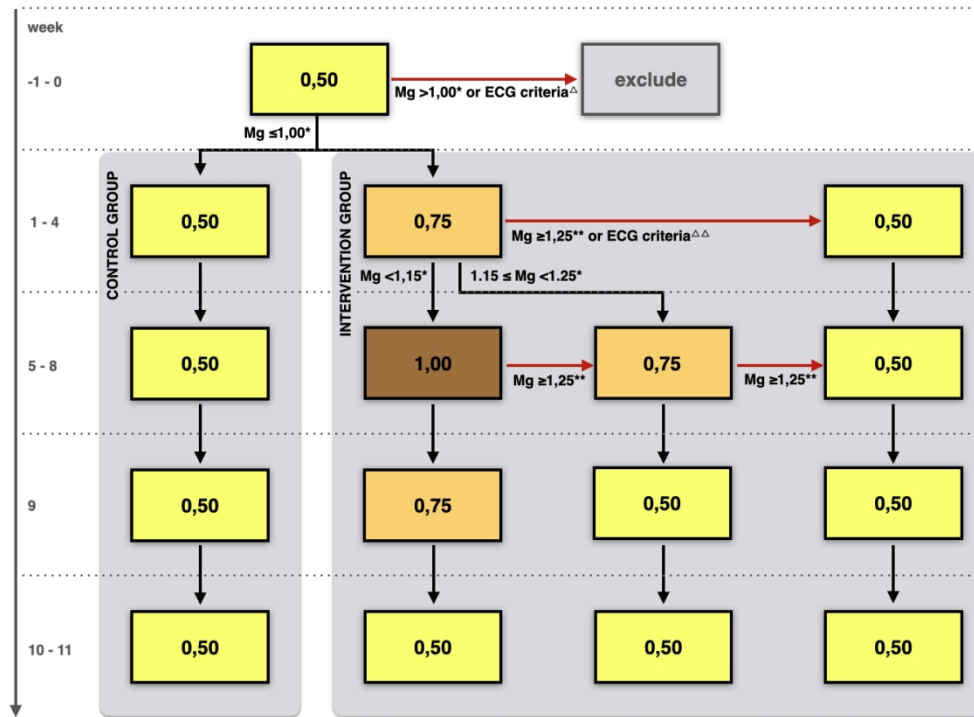
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For peer review only

## Figure legends

**Figure 1. Flow chart of the study intervention.** Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; <sup>△</sup>, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; <sup>△△</sup>, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; \*, pre-dialysis plasma Mg concentration after the long interdialytic interval; \*\*, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

**Figure 2. Study procedures.** dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.



**Figure 1. Flow chart of the study intervention.** Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; <sup>Δ</sup>, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; <sup>ΔΔ</sup>, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; \*, pre-dialysis plasma Mg concentration after the long interdialytic interval; \*\*, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

483x357mm (72 x 72 DPI)

Week	Dialysate	Blood sampling				ECG	Questionnaire	3DDD	Holter	PWV
		dialysis 1		dialysis 2						
		pre	post	pre	post	pre	post			
-1	standard	set 1	set 1	set 1	set 1			X		
0	standard								X	
1	study dialysate	set 3+A+B	set 2	set 1	set 1	set 1	set 1			
2	study dialysate	set 1	set 1							
3	study dialysate	set 1	set 1							
4	study dialysate	set 1	set 1					X	X	
5	study dialysate	set 3	set 2	set 1	set 1	set 1	set 1			
6	study dialysate	set 1	set 1							
7	study dialysate	set 1	set 1							
8	study dialysate	set 1	set 1					X	X	
9	study dialysate	set 3+A	set 2							X
10	standard	set 1	set 1							
11	standard	set 1	set 1							

**Figure 2. Study procedures.** dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

321x127mm (144 x 144 DPI)



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Abstract & 5
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page
	5b	Name and contact information for the trial sponsor	investigator initiated
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A



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	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
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**Introduction**

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5, 10

**Methods: Participants, interventions, and outcomes**

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-9

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4		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
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7		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
8				
9	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
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14	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
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17	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10-11
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21	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5-6
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### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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27	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
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32	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
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35	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
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Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial	10-11

**Methods: Data collection, management, and analysis**

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-8, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11

**Methods: Monitoring**

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4	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
5			N/A
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9		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
10			N/A
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12	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
13			8-9
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15	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
16			N/A
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19	<b>Ethics and dissemination</b>		
20			
21	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
22			5
23			
24	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
25			N/A
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29	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
30			5-7
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32		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
33			Figure 2
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35	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
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Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
<b>Appendices</b>			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7-8, Figure 2

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\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

# BMJ Open

**Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.**

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3 **Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a**  
4 **randomized controlled trial to determine feasibility and safety of using**  
5 **increased dialysate magnesium concentrations to increase plasma**  
6 **magnesium concentrations in people treated with hemodialysis.**  
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42 **Abbreviations**

43 AE, adverse event; CKD, chronic kidney disease; CRP, C-reactive protein; ECG,  
44 electrocardiography; PAC, premature atrial complex; PWV, pulse wave velocity; PTH,  
45 parathyroid hormone; PVC, premature ventricular complex; SOC, standard of care; SAE,  
46 serious adverse event; SPIRIT, Standard Protocol Items: Recommendation for Interventional  
47 Trials (SPIRIT)  
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## **Abstract**

### ***Introduction***

People treated with hemodialysis are at increased risk for all-cause and cardiovascular mortality. Plasma magnesium concentration has been inversely associated with these risks. Therefore, plasma magnesium may be a new modifiable risk factor and an increase of dialysate magnesium concentration may be an easy, safe and effective way to increase plasma magnesium concentrations. Detailed information on modulating dialysate magnesium concentrations is limited in literature. Primary objective of this study is to determine the safety and feasibility to increase plasma magnesium concentrations in people treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate.

### ***Methods and analysis***

In this randomized double blinded standard of care controlled trial, fifty-three persons treated with hemodialysis, will be randomly allocated 2:1 to either a step-wise individually titrated increase of dialysate magnesium concentration from 0.50 to 0.75 to 1.00 mmol/L during 8 weeks, or a standard dialysate magnesium concentration of 0.50 mmol/L. Other study measurements include dietary records, questionnaires, electrocardiography (ECG), Holter registration and pulse wave velocity. The primary endpoint is pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8. In addition, the predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored using linear mixed models. Safety endpoint is defined by the occurrence of hypermagnesemia above 1.25 mmol/L, or bradycardia or prolonged QTc interval detected on the ECG.

### ***Ethics and dissemination***

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center. The results of the study will be disseminated by publication in peer-reviewed scientific journals and presentation at national or international conferences in the field of interest.

### ***Trial registration number***

NTR 6568 / NL6393



### Strengths and limitations

- Blinding and randomization prevents bias occurring from differences in life-style between groups, and enables objective collection and processing of data
- Dialysate concentrations will be individually titrated based on individual plasma magnesium concentrations by an independent physician
- Not only the effects of dialysate magnesium concentrations on plasma magnesium concentrations will be determined, but also the factors that are predictive for these effects
- Major limitation is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to limited study duration

## Introduction

People with chronic kidney disease (CKD) including those treated with dialysis are at increased risk for all-cause and cardiovascular mortality.<sup>1</sup> This increased risk persists after adjustment for traditional cardiovascular risk factors, indicating that other kidney specific factors contribute to the cardiovascular risk.<sup>1</sup> Recently, lower magnesium has been identified as a potential novel risk factor.<sup>2</sup> Magnesium is involved in many physiological functions, including energy metabolism and regulation of transmembrane transport of ions and consequently, it is essential for muscle function, cardiac rhythm and vascular tone.<sup>3</sup> In a meta-analysis of studies in people with CKD including those on dialysis, we showed that plasma magnesium concentration is inversely associated with all-cause and cardiovascular mortality, and that this not only applies for normal compared to low magnesium, but also revealed protective effects of magnesium above as compared to within the reference range (generally 0.70 – 1.05 mmol/L).<sup>4</sup> Magnesium concentration is also inversely associated with the risk for sudden death and with arrhythmia in people treated with hemodialysis.<sup>5-7</sup> In the general population, serum magnesium has been inversely associated with frequent or complex premature complexes, which predict prognosis including all-cause mortality in the general population.<sup>8,9</sup> Moreover, serum magnesium was inversely associated with pulse wave velocity (PWV) in people treated with maintenance hemodialysis,<sup>10</sup> and in a short randomized cross-over study in people treated with hemodialysis, higher dialysate magnesium concentration compared to standard dialysate magnesium concentration decreased pulse wave velocity.<sup>11</sup> PWV is a marker of vascular stiffness and a strong predictor of cardiovascular outcome in people with CKD stage 4-5D.<sup>12</sup> In most studies that included multiple categories of plasma magnesium concentration in people with CKD including those treated with dialysis, there was a monotonic inverse association between magnesium and all-cause mortality.<sup>4</sup> We previously showed that a commonly used dialysate magnesium concentration of 0.50 mmol/L in hemodialysis, usually induces a decline of magnesium towards magnesium concentrations in the lower range of normal.<sup>13</sup> Therefore, an increase of dialysate magnesium concentration, may be an easy, safe and effective way to increase plasma magnesium concentrations in people treated with hemodialysis, without the need of oral supplementation. The results of one observational study suggest that there may be an optimal concentration of plasma magnesium in-between 1.15-1.27 mmol/L, with an increasing risk for mortality if magnesium values exceed this range.<sup>14</sup> Although these

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3 findings were not confirmed by other studies that included magnesium values in this high  
4 range, this requires to take into account safety when increasing plasma magnesium  
5 concentrations.<sup>6, 15, 16</sup> In a previous 4-weeks trial by Bressendorf et al., increasing dialysate  
6 magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase of  
7 plasma magnesium concentration (95%-CI 0.3-0.5).<sup>17</sup> Here, we describe a randomized  
8 standard-of-care controlled trial of step-wise increment of dialysate magnesium  
9 concentration in people treated with hemodialysis. Primary objective is to determine the  
10 feasibility to increase plasma magnesium concentrations in individuals treated with  
11 hemodialysis by means of sequentially increasing concentration of magnesium in the  
12 dialysate. Secondary objectives are to determine safety of using higher dialysate magnesium  
13 concentrations, the effect of dialysate magnesium on plasma magnesium concentration,  
14 and to define which parameters are predictive for the increment of plasma magnesium  
15 concentration by increasing dialysate magnesium. We will also explore the effects of using  
16 higher dialysate magnesium on cardiac rhythm and pulse wave velocity.  
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## 33 **Methods**

### 34 ***Trial design***

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36 In this randomized, double-blind, standard of care (SOC) controlled multi-center trial,  
37 individuals treated with hemodialysis will be randomly allocated to either a stepwise  
38 increase of dialysate magnesium concentration from 0.50 to 1.00 mmol/L, or continue on a  
39 standard dialysate magnesium concentration of 0.50 mmol/L. The protocol was written in  
40 accordance with the Standard Protocol Items: Recommendation for Interventional Trials  
41 (SPIRIT) and registered at [www.trialregister.nl](http://www.trialregister.nl) (registration number NTR 6568 / NL6393).<sup>18</sup>  
42  
43 The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and  
44 was approved by the Ethical Committee of the VU University Medical Center (registration  
45 number 2017.408, NL62679.029.17).  
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### 54 ***Characteristics of participants and recruitment***

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56 Adult persons treated with hemodialysis on a 3-times weekly dialysis schedule, will be  
57 enrolled in the study. The in- and exclusion criteria are listed in table 1. Participants will be  
58 recruited from multiple centers in the Netherlands, including Amsterdam University Medical  
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Center location VU Medical Center, Amsterdam; Diapriva dialysis center, Amsterdam; Niercentrum aan de Amstel, Amstelveen; and Spaarne Gasthuis, Hoofddorp. Participants need to provide written informed consent prior to enrollment.

**Table 1.** Inclusion and exclusion criteria

Inclusion criteria
<ul style="list-style-type: none"> <li>• Age <math>\geq 18</math> years</li> <li>• Hemodialysis with regular three times weekly dialysis schedule</li> <li>• Hemodialysis since at least 3 months</li> <li>• Standard dialysate magnesium concentration 0.50 mmol/L</li> <li>• Providing informed consent</li> <li>• Pre-dialysis plasma magnesium concentration <math>\leq 1.00</math> mmol/L after the long intra-dialytic interval</li> </ul>
Exclusion criteria
<ul style="list-style-type: none"> <li>• Intravenous magnesium supplementation (including total parenteral nutrition) in the last 2 weeks</li> <li>• Expected cessation of dialysis treatment within three months after inclusion or expected permanent or temporary dialysis center switch to a center not participating in the trial within three months after inclusion.</li> <li>• Prolongation of QTc interval: male <math>&gt;450</math> ms or female <math>&gt;460</math> ms on baseline ECG</li> <li>• Bradycardia: heart rate below 60 beats per minute on baseline ECG</li> <li>• Chronic arrhythmia or cardiac conduction disorder other than atrial fibrillation or ventricular extrasystole that poses the patient at risk at the discretion of the treating physician.</li> <li>• Change of proton pump inhibitor prescription in the last 2 weeks</li> </ul>

*In order to be eligible to participate in this study, a subject must meet all of the inclusion criteria. A potential subject who meets any of the exclusion criteria will be excluded from participation in this study.*

### **Intervention**

In the intervention group, dialysate magnesium is increased stepwise, from 0.50 mmol/L at baseline, to 0.75 mmol/L during week 1-4, and to 1.00 mmol/L during week 5-8. The participant proceeds to the next increment step of dialysate magnesium concentration after week 4 only if pre-dialysis plasma magnesium after the long interdialytic interval is below 1.15 mmol/L in week 4. Otherwise, the dialysate magnesium concentration of 0.75 mmol/L is continued in week 5-8. After week 8, dialysate magnesium will be gradually reduced with 0.25 mmol/L in week 9 and thereafter return to the standard dialysate magnesium concentration of 0.50 mmol/L in week 10.

Participants in the control group are treated with a standard dialysate magnesium concentration of 0.50 mmol/L. (see Figure 1)

The dialysate contains a potassium concentration of 2 or 3 mmol/L, as determined in routine care by the treating physician based on individual needs. For the respective

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3 magnesium concentrations, six dialysis concentrates are used in weeks 1-9 (Hemodialysis A-  
4 concentrate, D761, D987, D907, D283, D961 and D908, MTN Neubrandenburg GmbH,  
5 Neubrandenburg, Germany). In the mineral composition of these concentrates, besides  
6 potassium based on individual needs, only the amount of magnesium chloride is different.  
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8 Calcium concentration is these dialysates 1.25 mmol/L and the acidifier is acetate.”  
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#### 14 ***Study procedures and participant time line***

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16 The study procedures and participant time line are shown in Figure 2. After informed  
17 consent is provided by participants that meet in- and exclusion criteria, blood sampling and  
18 electrocardiography (ECG) are performed at baseline. Eligible persons meeting the criteria  
19 for plasma magnesium and ECG, as provided in table 1, are allocated to the either SOC or  
20 incremental magnesium dialysate. During the trial, blood sampling will be performed before  
21 and after the dialysis sessions following the long interdialytic interval weekly, and every  
22 dialysis session in week 1 and 5 to measure plasma magnesium concentration. In addition,  
23 in week 1, 5 and 9, blood is collected for measurements of potassium, bicarbonate, calcium,  
24 albumin, phosphate, parathyroid hormone (PTH), hemoglobin and C-reactive protein (CRP).  
25 For laboratory measurements, standard methods of the local laboratory are used.  
26  
27 Participants record dietary intake for 3-days at baseline. From this record, dietary  
28 magnesium intake is extracted using the Dutch Food Composition Database (NEVO) by using  
29 the calculator on the website of the Dutch Nutrition Center.<sup>19, 20</sup> A questionnaire regarding  
30 the presence of subjective symptoms that can be associated with hypermagnesemia is  
31 completed at baseline, week 4 and 8. This is a 7-point yes or no questionnaire to ask if a  
32 participant experienced the following symptoms in the last week: nausea, vomiting,  
33 dizziness, drowsiness, reduced muscle strength, itching and leg cramps, and at baseline, also  
34 a question about chronic diarrhea and over-the-counter use of magnesium supplements is  
35 included. An electrocardiogram (ECG) is repeated before dialysis in week 4 and 8 to  
36 determine heart rhythm, frequency and QTc interval. In addition, at baseline and in week 8,  
37 participants undergo continuous heart rhythm monitoring and pulse wave velocity (PWV)  
38 measurements. Heart rhythm monitoring is performed using a Holter recorder (Fysiologic,  
39 Amsterdam, The Netherlands) for 48 hours including one dialysis sessions and one  
40 interdialytic period. Carotid-femoral PWV is measured using the Sphygmocor tonometer  
41 (Atcor Medical Pty Ltd, Software version 9.0, Sydney, Australia) preceding the midweek  
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3 dialysis sessions. Participants are requested to avoid coffee, tea and smoking for 4 hours,  
4 and alcohol for 12 hours preceding the measurements, as is recommended by the  
5 manufacturer of the device. The patient is placed in supine position, in a quiet environment  
6 at room temperature. After attachment of ECG electrodes and bedrest for at least 5  
7 minutes, blood pressure is measured with an automated Omron device at least 3 times with  
8 a few minutes in-between, and until no substantial change occurs. Then, the last blood  
9 pressure measurement is recorded. The carotid to femoral artery distance is measured  
10 directly and multiplied by 0.8 to estimate the difference between cardiac-carotid and  
11 cardiac-femoral distance as recommended by expert consensus.<sup>21</sup> The pulse wave is  
12 recorded using the tonometer at the carotid and femoral site and then PWV is calculated  
13 automatically by the Sphygmocor software from entered blood pressure, distance and ECG  
14 and tonometer recordings. Measurements are performed at least twice or until two  
15 measurements are of appropriate quality. A measurement is considered of sufficient quality  
16 based on the criteria set by the manufacturer: adequate shape of detected signal of ECG  
17 and pulse wave, difference of heart rate  $\leq 5$  bpm between carotid and femoral  
18 measurement, ECG R-tops and pulse wave beginning are correctly identified by the  
19 software, and standard deviations of the ECG to carotid and of ECG to femoral time are both  
20  $< 6\%$ . PWV measurement is not performed in participants with irregular heart rhythm,  
21 pacemaker rhythm, atrial fibrillation or flutter, heart frequency below 40 or above 160 bpm,  
22 2<sup>nd</sup> or 3<sup>rd</sup> degree atrioventricular block, severe aortic valve stenosis or instable carotid  
23 plaque, as contra-indicated by the manufacturer. Furthermore, persons' characteristics are  
24 recorded at baseline and characteristics of the dialysis are recorded at baseline and weekly  
25 for the time of dialysis after the long interdialytic interval and for every dialysis during the  
26 first and fifth week of intervention. Recorded dialysis characteristics include modality  
27 (hemodialysis or hemodiafiltration), vascular access (catheter, fistula or graft), estimation of  
28 dialysis efficiency (Kt/Vurea per session according to Daugirdas' formula), treatment time  
29 per session, blood flow, dialysate flow and ultrafiltration volume. Medication use and  
30 dosage is recorded at baseline and at week 8. During the trial, all participants receive three  
31 times weekly hemodialysis sessions according to their regular schedule. Changes of dialysis  
32 schedule during the study will be avoided as much as possible if there is no medical  
33 indication to change the scheme. Also, changes in prescription of proton pump inhibitors  
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3 and magnesium-containing supplements, laxatives and phosphate binders will be avoided if  
4 clinically allowed.  
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### 8 ***Safety monitoring***

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10 If participants in the intervention group reach a plasma magnesium of 1.25 mmol/L or  
11 above, as noted by an unblinded independent nephrologist not involved in the trial (see  
12 below), at any time point during follow-up in week 1 to 8, dialysate magnesium  
13 concentration will be reduced to the previous level in the next week. If plasma magnesium  
14 remains 1.25 mmol/L or above in the next week in intervention phase 2 (week 5-8),  
15 dialysate magnesium is further reduced one step (to 0.50 mmol/L). If a participant develops  
16 bradycardia with heart rate below 50 beats per minute (bpm), or prolonged QTc interval  
17 (>450 ms in male or >460 ms in female), noticed on electrocardiography (ECG) in week 4,  
18 dialysate magnesium concentration is also reduced to the previous step. (Figure 1) Plasma  
19 magnesium above 1.25 mmol/L, bradycardia < 50 bpm and prolonged QTc will be recorded  
20 as adverse event (AE). Serious adverse events (SAE's) will be recorded and reported to the  
21 primary investigator and medical ethical committee.  
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### 34 ***Main endpoints***

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36 Primary endpoint is pre-dialysis plasma magnesium concentration after the long  
37 interdialytic interval at the end of week 8, in the intervention group compared to the  
38 control group. The change of pre-dialysis plasma magnesium concentration after the long  
39 interdialytic interval from baseline to the end of week 8, will also be determined in the  
40 intervention group compared to the control group.  
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### 47 ***Secondary endpoints***

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49 Post-dialysis plasma magnesium concentrations after the dialysis sessions following the long  
50 interdialytic interval will also be determined. Safety endpoint is the safety of using higher  
51 magnesium concentrations in the dialysate, as indicated by the incidence of respectively  
52 hypermagnesemia (>1.25 mmol/L) at any timepoint, or bradycardia (defined as heart rate  
53 below 60 bpm) or prolonged duration of QTc interval (>450ms in male or >460ms in female)  
54 identified on the ECG in week 4 or 8. Other explorative endpoints include the change of  
55 PWV from baseline to week 8; and the number of complex premature ventricular complexes  
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3 (complex PVC's), premature atrial complexes (PAC's), and heart rate variability as detected  
4 with Holter ECG monitoring. Complex PVC's are defined as PVC's that are multiform,  
5 repetitive or have a frequency of >30/h.<sup>8</sup> Furthermore, the following outcomes will be  
6 recorded: subjective symptoms that can be associated with hypermagnesemia determined  
7 from self-reporting in questionnaires in week 4 and 8; hospitalization; mortality; and  
8 cardiovascular events that lead to hospitalization or mortality including arrhythmia, cardiac  
9 arrest, acute coronary syndrome, cerebrovascular accident, and hemorrhage from ruptured  
10 aneurysms of the abdominal aorta.  
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### 20 ***Blinding and randomization***

21 Participants that fulfill all screening criteria, will be randomly allocated 2:1 in tranches of 6  
22 to either the intervention group or the control group by the pharmacy according to a  
23 computer-generated random list. Participants, treating physicians, nurses and researchers  
24 are blinded to treatment allocation. Dialysate cannisters are re-labelled by the pharmacy for  
25 the individual participant per individual study week. Labels include information on  
26 participant name, date of birth, study week and dialysate potassium concentration, but no  
27 further information on dialysate composition. For the first study week, the pharmacy  
28 chooses the appropriate dialysate concentrate based on treatment allocation and the in  
29 routine individual care determined potassium concentration. From week 2 on, one  
30 independent nephrologist that is not blinded for treatment allocation, weekly decides upon  
31 the dialysate magnesium concentration, after review of plasma magnesium concentrations  
32 according to the algorithm shown in Figure 1. The pharmacy then re-labels the dialysate as  
33 prescribed by the independent nephrologist.  
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### 48 ***Sample size calculation***

49 We previously performed a study to determine plasma magnesium concentrations and  
50 variability in people receiving 3-times weekly hemodialysis treatment with a standard  
51 dialysate magnesium concentration of 0.50 mmol/L.<sup>13</sup> That study showed a mean pre-  
52 dialysis plasma magnesium concentration of 0.88 ± 0.14 mmol/L.<sup>13</sup> After excluding pre-  
53 dialysis plasma magnesium levels above 1.00 mmol/L from the analysis, mean pre-dialysis plasma  
54 magnesium level was 0.83 mmol/L in that study population. Based on these results, we  
55 expect a mean plasma magnesium concentration of 0.83 ± 0.14 mmol/L in the control  
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3 group. Based on the results from the CONTRAST cohort analysis, in which plasma  
4 magnesium was associated with all-cause and cardiovascular mortality, we consider an  
5 increase of plasma magnesium concentration to 0.96 mmol/L in the intervention group  
6 relevant, which is a 0.13 mmol/L rise.<sup>5</sup> The required sample size calculated for two  
7 independent groups, based on the values just mentioned, a power of 0.80, probability of  
8 0.05, and 2:1 randomization, would be 28 in the intervention and 14 in the control group. To  
9 account for an estimated drop-out of 20%, the required sample size is 53 participants in  
10 total: 35 in the intervention and 18 in the control group.  
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### 20 **Statistical analysis**

21 Continuous variables will be expressed as mean and standard deviation (SD) for normally  
22 distributed variables or median and interquartile range (Q1-Q3) for non-parametric  
23 distributed variables. Categorical variables will be presented as number and percentage. The  
24 primary endpoint, pre-dialysis plasma magnesium after the long interdialytic interval at the  
25 end of week 8, will be compared between the intervention and control group, using  
26 univariable analysis with an unpaired Student's t-test if variables are normally distributed (if  
27 necessary after logarithmic transformation) and with Mann-Whitney U test if variables are  
28 non-normally distributed. The analysis is performed as intention to treat, including all  
29 participants that are still in study follow-up at week 8. The change of plasma magnesium  
30 from baseline to week 8, is first analyzed within each group using a paired Student's t-test,  
31 and then the difference from baseline to week 8 (delta) is compared between the groups  
32 using linear mixed models. The predictive effect of dialysate magnesium concentration and  
33 other baseline parameters and dialysis characteristics on plasma magnesium concentration,  
34 will be explored in two separate analyses (for pre-dialysis and post-dialysis concentration)  
35 using linear mixed models. In addition, we will explore which parameters are predictive for  
36 the increment of plasma magnesium concentration from baseline to week 8 after  
37 sequentially increasing dialysate magnesium concentration, using linear mixed models. For  
38 secondary endpoints, univariable analysis for within-group changes and between-group  
39 differences will be performed using respectively paired and unpaired Student's t-test or  
40 Mann-Whitney U tests for continuous variables, and chi-square or Fisher's exact test for  
41 dichotomous variables. Linear mixed models will be used for multivariable analysis of  
42 secondary endpoints with repeated measures.  
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### ***Data management***

Data is recorded in electronic case report forms using the webbased software Castor EDC (Amsterdam, The Netherlands). All data is stored in coded form. The identification key is stored at the local study site only. Randomization codes are stored at the pharmacy. The randomization code will not be broken until follow-up of all participants is completed.

### ***Dissemination***

The results of this study will be offered for publication in international peer-reviewed journals. In addition, the results can be presented at national and international conferences and meetings in the field.

### ***Patient and public involvement***

This study protocol was reviewed and approved by the Dutch Kidney Foundation (DKF). A patient panel of the Dutch Renal Patients Association is involved in the review of research protocols submitted to the DKF. Investigators will communicate results to participants once the final results become available. The results will also be shared with patient organizations.

### **Discussion**

This study aims to determine feasibility, safety, and predictive parameters for the effect of using dialysate with higher magnesium concentration to increase plasma magnesium concentrations in people treated with hemodialysis. In addition, this study will explore effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.

### ***Relation with previous studies***

In a previous study, we demonstrated that a commonly used dialysate magnesium concentration of 0.50 mmol/L generally induces a decline of plasma magnesium concentrations towards concentrations in the lower range of normal.<sup>13</sup> Detailed information in literature on the effects of increasing dialysate magnesium concentration on pre- and post-dialysis plasma magnesium concentrations and safety is sparse. Two other studies showed that a dialysate magnesium concentration of 0.75 mmol/L generally resulted in a

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3 relatively stable plasma magnesium concentration, with a mean pre-dialysis concentration  
4 of 1.2 mmol/L and mean post-dialysis concentrations of 1.1 up to 1.2 mmol/L.<sup>22, 23</sup> In  
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6 another trial, a 4-weeks single-step increment of dialysate magnesium concentration from  
7 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase (95%-CI 0.3-0.5) of pre-dialysis  
8  
9 plasma magnesium concentration and a mean pre-dialysis plasma Mg concentration of  $1.4 \pm$   
10  
11 0.2 mmol/L.<sup>17</sup> That study did not perform ECG nor Holter monitoring. As outlined in the  
12  
13 introduction, an observational study in people treated with hemodialysis found an inverse  
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15 association between plasma magnesium concentrations and arrhythmia.<sup>7</sup> Moreover, in a  
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17 short randomized cross-over study in people treated with hemodialysis, a dialysate  
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19 magnesium concentration of 0.75 mmol/L compared to 0.50 mmol/L decreased pulse wave  
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21 velocity.<sup>11</sup>  
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### 24 25 **Strengths and limitations**

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27 The strengths of this study protocol are the blinding and randomization. Although the  
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29 primary outcome is an objective outcome measure, blinding and randomization are  
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31 essential to prevent bias occurring from changes in life styles including dietary magnesium  
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33 intake. In addition, it is of relevance for objective collection and processing of data from  
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35 questionnaires, pulse wave velocity measurements, electrocardiography, and reporting of  
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37 SAE's. Another strength of this protocol is that dialysate magnesium concentration will be  
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39 individually titrated based on individual plasma magnesium concentrations. In addition, not  
40  
41 only the effect of dialysate magnesium will be determined, but also other factors that are  
42  
43 predictive for this effect will be determined. The major limitation of this protocol, is that the  
44  
45 study will not provide information on clinical outcomes including cardiovascular events and  
46  
47 mortality, due to a limited duration of the study.  
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### 49 **Potential impact**

50  
51 The study described in this protocol may provide relevant information on the effect of  
52  
53 dialysate magnesium on plasma magnesium concentrations, the strategy for titration of  
54  
55 dialysate magnesium based on individual needs, the safety of increasing plasma magnesium  
56  
57 concentrations, and about factors that are predictive for the effect of dialysate magnesium  
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59 on plasma magnesium concentration. This information may enable to safely increase plasma  
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magnesium concentrations by individualized dialysate magnesium concentrations. In

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3 addition, this study will provide explorative data about the effects of increased dialysate  
4 magnesium concentration on intermediate cardiovascular outcomes including cardiac  
5 rhythm and PWV. The information provided by this study, may pave the way to larger long-  
6 term randomized controlled trials on the effects of increasing plasma magnesium  
7 concentrations on clinical outcomes including all-cause and cardiovascular mortality in  
8 people treated with hemodialysis. If plasma magnesium indeed improves clinically relevant  
9 outcomes, and can be safely increased by means of individualized increasing dialysate  
10 magnesium concentrations, potentially large health benefits may be achieved if magnesium  
11 is increased slightly above the reference range by an increase of dialysate magnesium  
12 concentration. If so, the cost-effect ratio is likely low, as raising magnesium concentration is  
13 an inexpensive intervention. In addition, it would be an easy intervention that needs no  
14 additional patient effort and no oral supplementation would be needed in these persons  
15 that already experience a high pill burden. Therefore, the study described in this protocol  
16 may provide information of high relevance to patients, clinicians and health care providers  
17 and may eventually help to decrease morbidity and mortality in people treated with  
18 hemodialysis.  
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### 34 ***Safety considerations***

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36 Plasma magnesium concentrations are expected to rise up to values above the reference  
37 range by the increase of dialysate magnesium concentration. However, clinical symptoms of  
38 hypermagnesemia, typically are not observed before plasma magnesium concentrations  
39 exceed 2.0 mmol/L, which is high above the target concentrations in this study. Reported  
40 symptoms of hypermagnesemia (if plasma concentrations are above 2.0 mmol/L) include  
41 lethargy, drowsiness, flushing, nausea and vomiting, and diminished deep tendon  
42 reflexes.<sup>24</sup> In even more severe hypermagnesemia (plasma concentrations above 3.0  
43 mmol/L) also somnolence, loss of deep tendon reflexes, hypotension and ECG changes can  
44 occur, and in extreme hypermagnesemia (above 5.0 mmol/L) complete heart block, cardiac  
45 arrest, apnea, paralysis and coma have been reported<sup>24</sup>. As a result of hemodialysis inherent  
46 techniques, the increment of (free) plasma magnesium is restricted by its concentration in  
47 the dialysate (maximally 1.00 mmol/L in this study), so overshoot to symptomatic  
48 concentrations is virtually impossible. The risk for severe or symptomatic hypermagnesemia  
49 is further minimized by intensive monitoring. As dialysate magnesium concentration is not  
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3 further increased after a plasma magnesium concentration of 1.15 mmol/L is reached, and  
4 is reduced if plasma concentrations of 1.25 mmol/L are reached at any time point, severe or  
5 symptomatic hypermagnesemia will be prevented. These maximal target concentrations are  
6 set taking into account an observational study in people treated with hemodialysis that  
7 suggested an optimal magnesium concentration in-between 1.15-1.27 mmol/L and  
8 increased risk for mortality if magnesium exceeds 1.27 mmol/L.<sup>14</sup> Furthermore, for safety  
9 reasons, individuals with bradycardia or a prolonged QTc interval on the ECG at baseline will  
10 be excluded from participation, and in individuals with bradycardia with a heart rate below  
11 50 bpm or a prolonged QTc interval identified on the ECG in week 4, dialysate magnesium  
12 will be reduced. Based on these careful methods, the risk for individuals participating in this  
13 study is low. Considering the limited burden and risks associated with this study and a  
14 possible highly-relevant contribution to future improvement of treatment and prognosis in  
15 people treated with hemodialysis, the potential benefits outweigh the burden and possible  
16 risks.  
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### 30 **Trial status**

31 The trial is currently ongoing. The first participant was randomized on the 4th of April 2018  
32 and up till now 43 of 53 participants have been randomized.  
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### 39 **Funding**

40 This work was supported by the Dutch Kidney Foundation (PhD grant no. 15OP02) and the  
41 PPP Allowance made available by Top Sector Life Sciences & Health to The Dutch Kidney  
42 Foundation to stimulate public-private partnerships (grant no. LSHM17034-HSGF).  
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### 48 **Conflicts of interest statement**

49 MV received research grants from Vifor, Amgen, Fresenius, and acted as consultant for  
50 Medice, Astra Zeneca, Vifor, Amgen, Fresenius, Otsuma, Kyowa Kirin. The other authors  
51 declare no conflicts of interest.  
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### 57 **Authors contributions**

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3 NL, MV and JH conceived the study. NL, MV and CD designed the study. NL wrote the  
4 manuscript and MV, JH and CD revised the manuscript. Each author approved the final version  
5 of the manuscript.  
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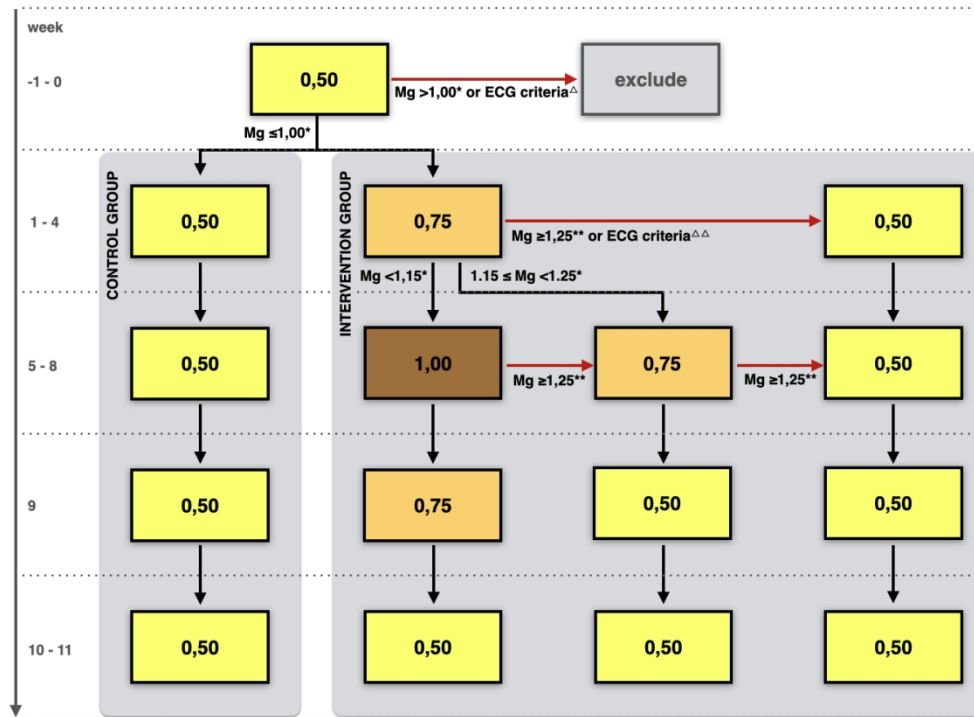
For peer review only



## Figure legends

**Figure 1. Flow chart of the study intervention.** Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography; <sup>△</sup>, ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female; <sup>△△</sup>, ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; \*, pre-dialysis plasma Mg concentration after the long interdialytic interval; \*\*, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

**Figure 2. Study procedures.** dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.



**Figure 1. Flow chart of the study intervention.** Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography;  $\Delta$ , ECG criteria at baseline: bradycardia defined as heart rate  $< 60$  bpm or prolonged QTc interval  $> 450$ ms in male or  $> 460$ ms in female;  $\Delta\Delta$ , ECG criteria in week 4: bradycardia with heart rate  $< 50$  bpm or prolonged QTc interval  $> 450$ ms in male or  $> 460$ ms in female; \*, pre-dialysis plasma Mg concentration after the long interdialytic interval; \*\*, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

483x357mm (72 x 72 DPI)

Week	Dialysate	Blood sampling				ECG	Questionnaire	3DDD	Holter	PWV
		dialysis 1		dialysis 2						
		pre	post	pre	post	pre	post			
-1	standard	set 1	set 1	set 1	set 1			X		
0	standard								X	
1	study dialysate	set 3+A+B	set 2	set 1	set 1	set 1	set 1			
2	study dialysate	set 1	set 1							
3	study dialysate	set 1	set 1							
4	study dialysate	set 1	set 1					X	X	
5	study dialysate	set 3	set 2	set 1	set 1	set 1	set 1			
6	study dialysate	set 1	set 1							
7	study dialysate	set 1	set 1							
8	study dialysate	set 1	set 1					X	X	
9	study dialysate	set 3+A	set 2							X
10	standard	set 1	set 1							
11	standard	set 1	set 1							

**Figure 2. Study procedures.** dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

321x127mm (144 x 144 DPI)



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Abstract & 5
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page
	5b	Name and contact information for the trial sponsor	investigator initiated
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A

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	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
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**Introduction**

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5, 10

**Methods: Participants, interventions, and outcomes**

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-9

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4		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
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7		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
8				
9	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
10				
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14	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
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17	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10-11
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21	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5-6
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### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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27	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
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32	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
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35	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
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Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial	10-11

**Methods: Data collection, management, and analysis**

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-8, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11

**Methods: Monitoring**

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4	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
5			N/A
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9		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
10			N/A
11			
12	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
13			8-9
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15	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
16			N/A
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19	<b>Ethics and dissemination</b>		
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21	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
22			5
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24	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
25			N/A
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29	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
30			5-7
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32		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
33			Figure 2
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35	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
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4	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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7	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
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10	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
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13	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
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18		31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
19				
20		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
21				
22				
23	<b>Appendices</b>			
24				
25	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
26				
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28				
29	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7-8, Figure 2
30				
31				

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

# BMJ Open

**Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a randomized controlled trial to determine feasibility and safety of using increased dialysate magnesium concentrations to increase plasma magnesium concentrations in people treated with hemodialysis.**

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3 **Magnesium in chronic hemodialysis (MAGIC-HD): a study protocol for a**  
4 **randomized controlled trial to determine feasibility and safety of using**  
5 **increased dialysate magnesium concentrations to increase plasma**  
6 **magnesium concentrations in people treated with hemodialysis.**  
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42 **Abbreviations**

43 AE, adverse event; CKD, chronic kidney disease; CRP, C-reactive protein; ECG,  
44 electrocardiography; PAC, premature atrial complex; PWV, pulse wave velocity; PTH,  
45 parathyroid hormone; PVC, premature ventricular complex; SOC, standard of care; SAE,  
46 serious adverse event; SPIRIT, Standard Protocol Items: Recommendation for Interventional  
47 Trials (SPIRIT)  
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## **Abstract**

### ***Introduction***

People treated with hemodialysis are at increased risk for all-cause and cardiovascular mortality. Plasma magnesium concentration has been inversely associated with these risks. Therefore, plasma magnesium may be a new modifiable risk factor and an increase of dialysate magnesium concentration may be an easy, safe and effective way to increase plasma magnesium concentrations. Detailed information on modulating dialysate magnesium concentrations is limited in literature. Primary objective of this study is to determine the safety and feasibility to increase plasma magnesium concentrations in people treated with hemodialysis by means of sequentially increasing concentration of magnesium in the dialysate.

### ***Methods and analysis***

In this randomized double blinded standard of care controlled trial, fifty-three persons treated with hemodialysis, will be randomly allocated 2:1 to either a step-wise individually titrated increase of dialysate magnesium concentration from 0.50 to 0.75 to 1.00 mmol/L during 8 weeks, or a standard dialysate magnesium concentration of 0.50 mmol/L. Other study measurements include dietary records, questionnaires, electrocardiography (ECG), Holter registration and pulse wave velocity. The primary endpoint is pre-dialysis plasma magnesium after the long interdialytic interval at the end of week 8. In addition, the predictive effect of dialysate magnesium concentration and other baseline parameters and dialysis characteristics on plasma magnesium concentration, will be explored using linear mixed models. Safety endpoint is defined by the occurrence of hypermagnesemia above 1.25 mmol/L, or bradycardia or prolonged QTc interval detected on the ECG.

### ***Ethics and dissemination***

The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and was approved by the Ethical Committee of the VU University Medical Center. The results of the study will be disseminated by publication in peer-reviewed scientific journals and presentation at national or international conferences in the field of interest.

### ***Trial registration number***

NTR 6568 / NL6393

### Strengths and limitations

- Blinding and randomization prevents bias occurring from differences in life-style between groups, and enables objective collection and processing of data
- Dialysate concentrations will be individually titrated based on individual plasma magnesium concentrations by an independent physician
- Not only the effects of dialysate magnesium concentrations on plasma magnesium concentrations will be determined, but also the factors that are predictive for these effects
- Major limitation is that the study will not provide information on clinical outcomes including cardiovascular events and mortality, due to limited study duration

## Introduction

People with chronic kidney disease (CKD) including those treated with dialysis are at increased risk for all-cause and cardiovascular mortality.<sup>1</sup> This increased risk persists after adjustment for traditional cardiovascular risk factors, indicating that other kidney specific factors contribute to the cardiovascular risk.<sup>1</sup> Recently, lower magnesium has been identified as a potential novel risk factor.<sup>2</sup> Magnesium is involved in many physiological functions, including energy metabolism and regulation of transmembrane transport of ions and consequently, it is essential for muscle function, cardiac rhythm and vascular tone.<sup>3</sup> In a meta-analysis of studies in people with CKD including those on dialysis, we showed that plasma magnesium concentration is inversely associated with all-cause and cardiovascular mortality, and that this not only applies for normal compared to low magnesium, but also revealed protective effects of magnesium above as compared to within the reference range (generally 0.70 – 1.05 mmol/L).<sup>4</sup> Magnesium concentration is also inversely associated with the risk for sudden death and with arrhythmia in people treated with hemodialysis.<sup>5-7</sup> In the general population, serum magnesium has been inversely associated with frequent or complex premature complexes, which predict prognosis including all-cause mortality in the general population.<sup>8,9</sup> Moreover, serum magnesium was inversely associated with pulse wave velocity (PWV) in people treated with maintenance hemodialysis,<sup>10</sup> and in a short randomized cross-over study in people treated with hemodialysis, higher dialysate magnesium concentration compared to standard dialysate magnesium concentration decreased pulse wave velocity.<sup>11</sup> PWV is a marker of vascular stiffness and a strong predictor of cardiovascular outcome in people with CKD stage 4-5D.<sup>12</sup> In most studies that included multiple categories of plasma magnesium concentration in people with CKD including those treated with dialysis, there was a monotonic inverse association between magnesium and all-cause mortality.<sup>4</sup> We previously showed that a commonly used dialysate magnesium concentration of 0.50 mmol/L in hemodialysis, usually induces a decline of magnesium towards magnesium concentrations in the lower range of normal.<sup>13</sup> Therefore, an increase of dialysate magnesium concentration, may be an easy, safe and effective way to increase plasma magnesium concentrations in people treated with hemodialysis, without the need of oral supplementation. The results of one observational study suggest that there may be an optimal concentration of plasma magnesium in-between 1.15-1.27 mmol/L, with an increasing risk for mortality if magnesium values exceed this range.<sup>14</sup> Although these

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3 findings were not confirmed by other studies that included magnesium values in this high  
4 range, this requires to take into account safety when increasing plasma magnesium  
5 concentrations.<sup>6, 15, 16</sup> In a previous 4-weeks trial by Bressendorf et al., increasing dialysate  
6 magnesium concentration from 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase of  
7 plasma magnesium concentration (95%-CI 0.3-0.5).<sup>17</sup> Here, we describe a randomized  
8 standard-of-care controlled trial of step-wise increment of dialysate magnesium  
9 concentration in people treated with hemodialysis. Primary objective is to determine the  
10 feasibility to increase plasma magnesium concentrations in individuals treated with  
11 hemodialysis by means of sequentially increasing concentration of magnesium in the  
12 dialysate. Secondary objectives are to determine safety of using higher dialysate magnesium  
13 concentrations, the effect of dialysate magnesium on plasma magnesium concentration,  
14 and to define which parameters are predictive for the increment of plasma magnesium  
15 concentration by increasing dialysate magnesium. We will also explore the effects of using  
16 higher dialysate magnesium on cardiac rhythm and pulse wave velocity.  
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## 33 **Methods**

### 34 ***Trial design***

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36 In this randomized, double-blind, standard of care (SOC) controlled multi-center trial,  
37 individuals treated with hemodialysis will be randomly allocated to either a stepwise  
38 increase of dialysate magnesium concentration from 0.50 to 1.00 mmol/L, or continue on a  
39 standard dialysate magnesium concentration of 0.50 mmol/L. The protocol was written in  
40 accordance with the Standard Protocol Items: Recommendation for Interventional Trials  
41 (SPIRIT) and originally prospectively registered at [www.trialregister.nl](http://www.trialregister.nl), which is now  
42 included in the International Clinical Trial Registry Platform (ICTRP) and can be accessed via  
43 <https://trialssearch.who.int> (registration number NTR 6568 / NL6393).<sup>18</sup> The first participant  
44 was randomized in April 2018 and ending of the study is expected at the end of December  
45 2022.  
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### 54 ***Characteristics of participants and recruitment***

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56 Adult persons treated with hemodialysis on a 3-times weekly dialysis schedule, will be  
57 enrolled in the study. The in- and exclusion criteria are listed in table 1. Participants will be  
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recruited from multiple centers in the Netherlands, including Amsterdam University Medical Center location VU Medical Center, Amsterdam; Diapriva dialysis center, Amsterdam; Niercentrum aan de Amstel, Amstelveen; and Spaarne Gasthuis, Hoofddorp. Participants need to provide written informed consent prior to enrollment.

**Table 1.** Inclusion and exclusion criteria

Inclusion criteria
<ul style="list-style-type: none"> <li>• Age <math>\geq 18</math> years</li> <li>• Hemodialysis with regular three times weekly dialysis schedule</li> <li>• Hemodialysis since at least 3 months</li> <li>• Standard dialysate magnesium concentration 0.50 mmol/L</li> <li>• Providing informed consent</li> <li>• Pre-dialysis plasma magnesium concentration <math>\leq 1.00</math> mmol/L after the long intra-dialytic interval</li> </ul>
Exclusion criteria
<ul style="list-style-type: none"> <li>• Intravenous magnesium supplementation (including total parenteral nutrition) in the last 2 weeks</li> <li>• Expected cessation of dialysis treatment within three months after inclusion or expected permanent or temporary dialysis center switch to a center not participating in the trial within three months after inclusion.</li> <li>• Prolongation of QTc interval: male <math>&gt;450</math> ms or female <math>&gt;460</math> ms on baseline ECG</li> <li>• Bradycardia: heart rate below 60 beats per minute on baseline ECG</li> <li>• Chronic arrhythmia or cardiac conduction disorder other than atrial fibrillation or ventricular extrasystole that poses the patient at risk at the discretion of the treating physician.</li> <li>• Change of proton pump inhibitor prescription in the last 2 weeks</li> </ul>

*In order to be eligible to participate in this study, a subject must meet all of the inclusion criteria. A potential subject who meets any of the exclusion criteria will be excluded from participation in this study.*

### **Intervention**

In the intervention group, dialysate magnesium is increased stepwise, from 0.50 mmol/L at baseline, to 0.75 mmol/L during week 1-4, and to 1.00 mmol/L during week 5-8. The participant proceeds to the next increment step of dialysate magnesium concentration after week 4 only if pre-dialysis plasma magnesium after the long interdialytic interval is below 1.15 mmol/L in week 4. Otherwise, the dialysate magnesium concentration of 0.75 mmol/L is continued in week 5-8. After week 8, dialysate magnesium will be gradually reduced with 0.25 mmol/L in week 9 and thereafter return to the standard dialysate magnesium concentration of 0.50 mmol/L in week 10.

Participants in the control group are treated with a standard dialysate magnesium concentration of 0.50 mmol/L. (see Figure 1)



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3 The dialysate contains a potassium concentration of 2 or 3 mmol/L, as determined in  
4 routine care by the treating physician based on individual needs. For the respective  
5 magnesium concentrations, six dialysis concentrates are used in weeks 1-9 (Hemodialysis A-  
6 concentrate, D761, D987, D907, D283, D961 and D908, MTN Neubrandenburg GmbH,  
7 Neubrandenburg, Germany). In the mineral composition of these concentrates, besides  
8 potassium based on individual needs, only the amount of magnesium chloride is different.  
9 Calcium concentration is these dialysates 1.25 mmol/L and the acidifier is acetate.  
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### 18 ***Study procedures and participant time line***

19 The study procedures and participant time line are shown in Figure 2. After informed  
20 consent is provided by participants that meet in- and exclusion criteria, blood sampling and  
21 electrocardiography (ECG) are performed at baseline. Eligible persons meeting the criteria  
22 for plasma magnesium and ECG, as provided in table 1, are allocated to the either SOC or  
23 incremental magnesium dialysate. During the trial, blood sampling will be performed before  
24 and after the dialysis sessions following the long interdialytic interval weekly, and every  
25 dialysis session in week 1 and 5 to measure plasma magnesium concentration. In addition,  
26 in week 1, 5 and 9, blood is collected for measurements of potassium, bicarbonate, calcium,  
27 albumin, phosphate, parathyroid hormone (PTH), hemoglobin and C-reactive protein (CRP).  
28 For laboratory measurements, standard methods of the local laboratory are used.  
29 Participants record dietary intake for 3-days at baseline. From this record, dietary  
30 magnesium intake is extracted using the Dutch Food Composition Database (NEVO) by using  
31 the calculator on the website of the Dutch Nutrition Center.<sup>19, 20</sup> A questionnaire regarding  
32 the presence of subjective symptoms that can be associated with hypermagnesemia is  
33 completed at baseline, week 4 and 8. This is a 7-point yes or no questionnaire to ask if a  
34 participant experienced the following symptoms in the last week: nausea, vomiting,  
35 dizziness, drowsiness, reduced muscle strength, itching and leg cramps, and at baseline, also  
36 a question about chronic diarrhea and over-the-counter use of magnesium supplements is  
37 included. An electrocardiogram (ECG) is repeated before dialysis in week 4 and 8 to  
38 determine heart rhythm, frequency and QTc interval. In addition, at baseline and in week 8,  
39 participants undergo continuous heart rhythm monitoring and pulse wave velocity (PWV)  
40 measurements. Heart rhythm monitoring is performed using a Holter recorder (Fysiologic,  
41 Amsterdam, The Netherlands) for 48 hours including one dialysis sessions and one  
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3 interdialytic period. Carotid-femoral PWV is measured using the Sphygmocor tonometer  
4 (Atcor Medical Pty Ltd, Software version 9.0, Sydney, Australia) preceding the midweek  
5 dialysis sessions. Participants are requested to avoid coffee, tea and smoking for 4 hours,  
6 and alcohol for 12 hours preceding the measurements, as is recommended by the  
7 manufacturer of the device. The patient is placed in supine position, in a quiet environment  
8 at room temperature. After attachment of ECG electrodes and bedrest for at least 5  
9 minutes, blood pressure is measured with an automated Omron device at least 3 times with  
10 a few minutes in-between, and until no substantial change occurs. Then, the last blood  
11 pressure measurement is recorded. The carotid to femoral artery distance is measured  
12 directly and multiplied by 0.8 to estimate the difference between cardiac-carotid and  
13 cardiac-femoral distance as recommended by expert consensus.<sup>21</sup> The pulse wave is  
14 recorded using the tonometer at the carotid and femoral site and then PWV is calculated  
15 automatically by the Sphygmocor software from entered blood pressure, distance and ECG  
16 and tonometer recordings. Measurements are performed at least twice or until two  
17 measurements are of appropriate quality. A measurement is considered of sufficient quality  
18 based on the criteria set by the manufacturer: adequate shape of detected signal of ECG  
19 and pulse wave, difference of heart rate  $\leq 5$  bpm between carotid and femoral  
20 measurement, ECG R-tops and pulse wave beginning are correctly identified by the  
21 software, and standard deviations of the ECG to carotid and of ECG to femoral time are both  
22  $< 6\%$ . PWV measurement is not performed in participants with irregular heart rhythm,  
23 pacemaker rhythm, atrial fibrillation or flutter, heart frequency below 40 or above 160 bpm,  
24 2<sup>nd</sup> or 3<sup>rd</sup> degree atrioventricular block, severe aortic valve stenosis or instable carotid  
25 plaque, as contra-indicated by the manufacturer. Furthermore, persons' characteristics are  
26 recorded at baseline and characteristics of the dialysis are recorded at baseline and weekly  
27 for the time of dialysis after the long interdialytic interval and for every dialysis during the  
28 first and fifth week of intervention. Recorded dialysis characteristics include modality  
29 (hemodialysis or hemodiafiltration), vascular access (catheter, fistula or graft), estimation of  
30 dialysis efficiency (Kt/Vurea per session according to Daugirdas' formula), treatment time  
31 per session, blood flow, dialysate flow and ultrafiltration volume. Medication use and  
32 dosage is recorded at baseline and at week 8. During the trial, all participants receive three  
33 times weekly hemodialysis sessions according to their regular schedule. Changes of dialysis  
34 schedule during the study will be avoided as much as possible if there is no medical  
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3 indication to change the scheme. Also, changes in prescription of proton pump inhibitors  
4 and magnesium-containing supplements, laxatives and phosphate binders will be avoided if  
5 clinically allowed.  
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### 10 ***Safety monitoring***

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12 If participants in the intervention group reach a plasma magnesium of 1.25 mmol/L or  
13 above, as noted by an unblinded independent nephrologist not involved in the trial (see  
14 below), at any time point during follow-up in week 1 to 8, dialysate magnesium  
15 concentration will be reduced to the previous level in the next week. If plasma magnesium  
16 remains 1.25 mmol/L or above in the next week in intervention phase 2 (week 5-8),  
17 dialysate magnesium is further reduced one step (to 0.50 mmol/L). If a participant develops  
18 bradycardia with heart rate below 50 beats per minute (bpm), or prolonged QTc interval  
19 (>450 ms in male or >460 ms in female), noticed on electrocardiography (ECG) in week 4,  
20 dialysate magnesium concentration is also reduced to the previous step. (Figure 1) Plasma  
21 magnesium above 1.25 mmol/L, bradycardia < 50 bpm and prolonged QTc will be recorded  
22 as adverse event (AE). Serious adverse events (SAE's) will be recorded and reported to the  
23 primary investigator and medical ethical committee.  
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### 36 ***Main endpoints***

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38 Primary endpoint is pre-dialysis plasma magnesium concentration after the long  
39 interdialytic interval at the end of week 8, in the intervention group compared to the  
40 control group. The change of pre-dialysis plasma magnesium concentration after the long  
41 interdialytic interval from baseline to the end of week 8, will also be determined in the  
42 intervention group compared to the control group.  
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### 49 ***Secondary endpoints***

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51 Post-dialysis plasma magnesium concentrations after the dialysis sessions following the long  
52 interdialytic interval will also be determined. Safety endpoint is the safety of using higher  
53 magnesium concentrations in the dialysate, as indicated by the incidence of respectively  
54 hypermagnesemia (>1.25 mmol/L) at any timepoint, or bradycardia (defined as heart rate  
55 below 60 bpm) or prolonged duration of QTc interval (>450ms in male or >460ms in female)  
56 identified on the ECG in week 4 or 8. Other explorative endpoints include the change of  
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3 PWV from baseline to week 8; and the number of complex premature ventricular complexes  
4 (complex PVC's), premature atrial complexes (PAC's), and heart rate variability as detected  
5 with Holter ECG monitoring. Complex PVC's are defined as PVC's that are multiform,  
6 repetitive or have a frequency of >30/h.<sup>8</sup> Furthermore, the following outcomes will be  
7 recorded: subjective symptoms that can be associated with hypermagnesemia determined  
8 from self-reporting in questionnaires in week 4 and 8; hospitalization; mortality; and  
9 cardiovascular events that lead to hospitalization or mortality including arrhythmia, cardiac  
10 arrest, acute coronary syndrome, cerebrovascular accident, and hemorrhage from ruptured  
11 aneurysms of the abdominal aorta.  
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### ***Blinding and randomization***

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23 Participants that fulfill all screening criteria, will be randomly allocated 2:1 in tranches of 6  
24 to either the intervention group or the control group by the pharmacy according to a  
25 computer-generated random list. Participants, treating physicians, nurses and researchers  
26 are blinded to treatment allocation. Dialysate cannisters are re-labelled by the pharmacy for  
27 the individual participant per individual study week. Labels include information on  
28 participant name, date of birth, study week and dialysate potassium concentration, but no  
29 further information on dialysate composition. For the first study week, the pharmacy  
30 chooses the appropriate dialysate concentrate based on treatment allocation and the in  
31 routine individual care determined potassium concentration. From week 2 on, one  
32 independent nephrologist that is not blinded for treatment allocation, weekly decides upon  
33 the dialysate magnesium concentration, after review of plasma magnesium concentrations  
34 according to the algorithm shown in Figure 1. The pharmacy then re-labels the dialysate as  
35 prescribed by the independent nephrologist.  
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### ***Sample size calculation***

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50 We previously performed a study to determine plasma magnesium concentrations and  
51 variability in people receiving 3-times weekly hemodialysis treatment with a standard  
52 dialysate magnesium concentration of 0.50 mmol/L.<sup>13</sup> That study showed a mean pre-  
53 dialysis plasma magnesium concentration of 0.88 ± 0.14 mmol/L.<sup>13</sup> After excluding pre-  
54 dialysis magnesium levels above 1.00 mmol/L from the analysis, mean pre-dialysis plasma  
55 magnesium level was 0.83 mmol/L in that study population. Based on these results, we  
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3 expect a mean plasma magnesium concentration of  $0.83 \pm 0.14$  mmol/L in the control  
4 group. Based on the results from the CONTRAST cohort analysis, in which plasma  
5 magnesium was associated with all-cause and cardiovascular mortality, we consider an  
6 increase of plasma magnesium concentration to 0.96 mmol/L in the intervention group  
7 relevant, which is a 0.13 mmol/L rise.<sup>5</sup> The required sample size calculated for two  
8 independent groups, based on the values just mentioned, a power of 0.80, probability of  
9 0.05, and 2:1 randomization, would be 28 in the intervention and 14 in the control group. To  
10 account for an estimated drop-out of 20%, the required sample size is 53 participants in  
11 total: 35 in the intervention and 18 in the control group.  
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### 22 ***Statistical analysis***

23 Continuous variables will be expressed as mean and standard deviation (SD) for normally  
24 distributed variables or median and interquartile range (Q1-Q3) for non-parametric  
25 distributed variables. Categorical variables will be presented as number and percentage. The  
26 primary endpoint, pre-dialysis plasma magnesium after the long interdialytic interval at the  
27 end of week 8, will be compared between the intervention and control group, using  
28 univariable analysis with an unpaired Student's t-test if variables are normally distributed (if  
29 necessary after logarithmic transformation) and with Mann-Whitney U test if variables are  
30 non-normally distributed. The analysis is performed as intention to treat, including all  
31 participants that are still in study follow-up at week 8. The change of plasma magnesium  
32 from baseline to week 8, is first analyzed within each group using a paired Student's t-test,  
33 and then the difference from baseline to week 8 (delta) is compared between the groups  
34 using linear mixed models. The predictive effect of dialysate magnesium concentration and  
35 other baseline parameters and dialysis characteristics on plasma magnesium concentration,  
36 will be explored in two separate analyses (for pre-dialysis and post-dialysis concentration)  
37 using linear mixed models. In addition, we will explore which parameters are predictive for  
38 the increment of plasma magnesium concentration from baseline to week 8 after  
39 sequentially increasing dialysate magnesium concentration, using linear mixed models. For  
40 secondary endpoints, univariable analysis for within-group changes and between-group  
41 differences will be performed using respectively paired and unpaired Student's t-test or  
42 Mann-Whitney U tests for continuous variables, and chi-square or Fisher's exact test for  
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3 dichotomous variables. Linear mixed models will be used for multivariable analysis of  
4 secondary endpoints with repeated measures.  
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### 8 ***Data management***

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10 Data is recorded in electronic case report forms using the webbased software Castor EDC  
11 (Amsterdam, The Netherlands). All data is stored in coded form. The identification key is  
12 stored at the local study site only. Randomization codes are stored at the pharmacy. The  
13 randomization code will not be broken until follow-up of all participants is completed.  
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### 18 ***Ethics and dissemination***

19  
20 The study is conducted in accordance with the declaration of Helsinki as revised in 2013 and  
21 was approved by the Ethical Committee of the VU University Medical Center (registration  
22 number 2017.408, NL62679.029.17).  
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26 The results of this study will be offered for publication in international peer-reviewed  
27 journals. In addition, the results can be presented at national and international conferences  
28 and meetings in the field.  
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### 33 ***Patient and public involvement***

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35 This study protocol was reviewed and approved by the Dutch Kidney Foundation (DKF). A  
36 patient panel of the Dutch Renal Patients Association is involved in the review of research  
37 protocols submitted to the DKF. Investigators will communicate results to participants once  
38 the final results become available. The results will also be shared with patient organizations.  
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### 47 **Discussion**

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49 This study aims to determine feasibility, safety, and predictive parameters for the effect of  
50 using dialysate with higher magnesium concentration to increase plasma magnesium  
51 concentrations in people treated with hemodialysis. In addition, this study will explore  
52 effects of using higher dialysate magnesium on cardiac rhythm and pulse wave velocity.  
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### 58 ***Relation with previous studies***

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3 In a previous study, we demonstrated that a commonly used dialysate magnesium  
4 concentration of 0.50 mmol/L generally induces a decline of plasma magnesium  
5 concentrations towards concentrations in the lower range of normal.<sup>13</sup> Detailed information  
6 in literature on the effects of increasing dialysate magnesium concentration on pre- and  
7 post-dialysis plasma magnesium concentrations and safety is sparse. Two other studies  
8 showed that a dialysate magnesium concentration of 0.75 mmol/L generally resulted in a  
9 relatively stable plasma magnesium concentration, with a mean pre-dialysis concentration  
10 of 1.2 mmol/L and mean post-dialysis concentrations of 1.1 up to 1.2 mmol/L.<sup>22, 23</sup> In  
11 another trial, a 4-weeks single-step increment of dialysate magnesium concentration from  
12 0.50 to 1.00 mmol/L resulted in a 0.4 mmol/L increase (95%-CI 0.3-0.5) of pre-dialysis  
13 plasma magnesium concentration and a mean pre-dialysis plasma Mg concentration of 1.4 ±  
14 0.2 mmol/L.<sup>17</sup> That study did not perform ECG nor Holter monitoring. As outlined in the  
15 introduction, an observational study in people treated with hemodialysis found an inverse  
16 association between plasma magnesium concentrations and arrhythmia.<sup>7</sup> Moreover, in a  
17 short randomized cross-over study in people treated with hemodialysis, a dialysate  
18 magnesium concentration of 0.75 mmol/L compared to 0.50 mmol/L decreased pulse wave  
19 velocity.<sup>11</sup>

### 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 **Strengths and limitations**

37 The strengths of this study protocol are the blinding and randomization. Although the  
38 primary outcome is an objective outcome measure, blinding and randomization are  
39 essential to prevent bias occurring from changes in life styles including dietary magnesium  
40 intake. In addition, it is of relevance for objective collection and processing of data from  
41 questionnaires, pulse wave velocity measurements, electrocardiography, and reporting of  
42 SAE's. Another strength of this protocol is that dialysate magnesium concentration will be  
43 individually titrated based on individual plasma magnesium concentrations. In addition, not  
44 only the effect of dialysate magnesium will be determined, but also other factors that are  
45 predictive for this effect will be determined. The major limitation of this protocol, is that the  
46 study will not provide information on clinical outcomes including cardiovascular events and  
47 mortality, due to a limited duration of the study.

### 48 49 50 51 52 53 54 55 56 57 58 59 60 **Potential impact**

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3 The study described in this protocol may provide relevant information on the effect of  
4 dialysate magnesium on plasma magnesium concentrations, the strategy for titration of  
5 dialysate magnesium based on individual needs, the safety of increasing plasma magnesium  
6 concentrations, and about factors that are predictive for the effect of dialysate magnesium  
7 concentrations, and about factors that are predictive for the effect of dialysate magnesium  
8 on plasma magnesium concentration. This information may enable to safely increase plasma  
9 magnesium concentrations by individualized dialysate magnesium concentrations. In  
10 addition, this study will provide explorative data about the effects of increased dialysate  
11 magnesium concentration on intermediate cardiovascular outcomes including cardiac  
12 rhythm and PWV. The information provided by this study, may pave the way to larger long-  
13 term randomized controlled trials on the effects of increasing plasma magnesium  
14 concentrations on clinical outcomes including all-cause and cardiovascular mortality in  
15 people treated with hemodialysis. If plasma magnesium indeed improves clinically relevant  
16 outcomes, and can be safely increased by means of individualized increasing dialysate  
17 magnesium concentrations, potentially large health benefits may be achieved if magnesium  
18 is increased slightly above the reference range by an increase of dialysate magnesium  
19 concentration. If so, the cost-effect ratio is likely low, as raising magnesium concentration is  
20 an inexpensive intervention. In addition, it would be an easy intervention that needs no  
21 additional patient effort and no oral supplementation would be needed in these persons  
22 that already experience a high pill burden. Therefore, the study described in this protocol  
23 may provide information of high relevance to patients, clinicians and health care providers  
24 and may eventually help to decrease morbidity and mortality in people treated with  
25 hemodialysis.

### 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 **Safety considerations**

46 Plasma magnesium concentrations are expected to rise up to values above the reference  
47 range by the increase of dialysate magnesium concentration. However, clinical symptoms of  
48 hypermagnesemia, typically are not observed before plasma magnesium concentrations  
49 exceed 2.0 mmol/L, which is high above the target concentrations in this study. Reported  
50 symptoms of hypermagnesemia (if plasma concentrations are above 2.0 mmol/L) include  
51 lethargy, drowsiness, flushing, nausea and vomiting , and diminished deep tendon  
52 reflexes.<sup>24</sup> In even more severe hypermagnesemia (plasma concentrations above 3.0  
53 mmol/L) also somnolence, loss of deep tendon reflexes, hypotension and ECG changes can  
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3 occur, and in extreme hypermagnesemia (above 5.0 mmol/L) complete heart block, cardiac  
4 arrest, apnea, paralysis and coma have been reported<sup>24</sup>. As a result of hemodialysis inherent  
5 techniques, the increment of (free) plasma magnesium is restricted by its concentration in  
6 the dialysate (maximally 1.00 mmol/L in this study), so overshoot to symptomatic  
7 concentrations is virtually impossible. The risk for severe or symptomatic hypermagnesemia  
8 is further minimized by intensive monitoring. As dialysate magnesium concentration is not  
9 further increased after a plasma magnesium concentration of 1.15 mmol/L is reached, and  
10 is reduced if plasma concentrations of 1.25 mmol/L are reached at any time point, severe or  
11 symptomatic hypermagnesemia will be prevented. These maximal target concentrations are  
12 set taking into account an observational study in people treated with hemodialysis that  
13 suggested an optimal magnesium concentration in-between 1.15-1.27 mmol/L and  
14 increased risk for mortality if magnesium exceeds 1.27 mmol/L.<sup>14</sup> Furthermore, for safety  
15 reasons, individuals with bradycardia or a prolonged QTc interval on the ECG at baseline will  
16 be excluded from participation, and in individuals with bradycardia with a heart rate below  
17 50 bpm or a prolonged QTc interval identified on the ECG in week 4, dialysate magnesium  
18 will be reduced. Based on these careful methods, the risk for individuals participating in this  
19 study is low. Considering the limited burden and risks associated with this study and a  
20 possible highly-relevant contribution to future improvement of treatment and prognosis in  
21 people treated with hemodialysis, the potential benefits outweigh the burden and possible  
22 risks.

### ***Trial status***

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43 The trial is currently ongoing. The first participant was randomized on the 4th of April 2018  
44 and up till now 43 of 53 participants have been randomized.

### **Funding**

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51 This work was supported by the Dutch Kidney Foundation (PhD grant no. 15OP02) and the  
52 PPP Allowance made available by Top Sector Life Sciences & Health to The Dutch Kidney  
53 Foundation to stimulate public-private partnerships (grant no. LSHM17034-HSGF).  
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### **Conflicts of interest statement**

1  
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3 MV received research grants from Vifor, Amgen, Fresenius, and acted as consultant for  
4 Medice, Astra Zeneca, Vifor, Amgen, Fresenius, Otsuma, Kyowa Kirin. The other authors  
5 declare no conflicts of interest.  
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### 10 **Authors contributions**

11 NL, MV and JH conceived the study. NL, MV and CD designed the study. NL wrote the  
12 manuscript and MV, JH and CD revised the manuscript. Each author approved the final version  
13 of the manuscript.  
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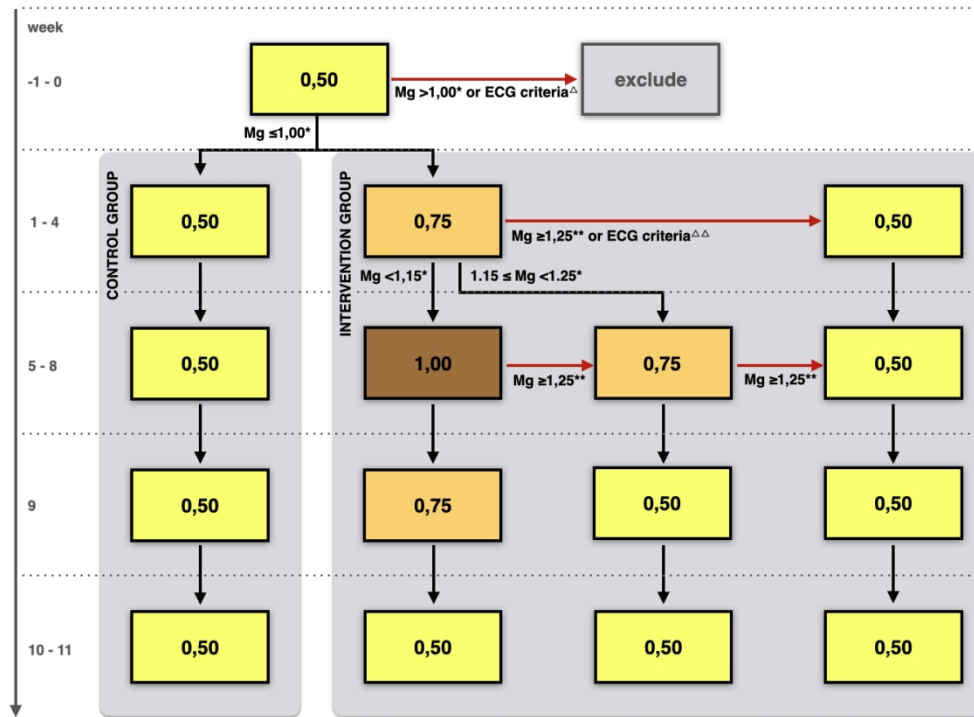
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## Figure legends

**Figure 1. Flow chart of the study intervention.** Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography;  $\Delta$ , ECG criteria at baseline: bradycardia defined as heartrate <60 bpm or prolonged QTc interval >450ms in male or >460ms in female;  $\Delta\Delta$ , ECG criteria in week 4: bradycardia with heart rate <50 bpm or prolonged QTc interval >450ms in male or >460ms in female; \*, pre-dialysis plasma Mg concentration after the long interdialytic interval; \*\*, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

**Figure 2. Study procedures.** dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.



**Figure 1. Flow chart of the study intervention.** Numbers within frames represent dialysate magnesium concentrations in mmol/L. All other magnesium concentrations represent plasma magnesium concentrations, also in mmol/L. Mg, magnesium; ECG; electrocardiography;  $^\Delta$ , ECG criteria at baseline: bradycardia defined as heartrate  $< 60$  bpm or prolonged QTc interval  $> 450$ ms in male or  $> 460$ ms in female;  $^{\Delta\Delta}$ , ECG criteria in week 4: bradycardia with heart rate  $< 50$  bpm or prolonged QTc interval  $> 450$ ms in male or  $> 460$ ms in female; \*, pre-dialysis plasma Mg concentration after the long interdialytic interval; \*\*, plasma Mg concentration at any time point (pre-dialysis and post-dialysis measurements included).

483x357mm (72 x 72 DPI)

Week	Dialysate	Blood sampling				ECG	Questionnaire	3DDD	Holter	PWV
		dialysis 1		dialysis 2						
		pre	post	pre	post	pre	post			
-1	standard	set 1	set 1	set 1	set 1			X		
0	standard								X	X
1	study dialysate	set 3+A+B	set 2	set 1	set 1	set 1	set 1			
2	study dialysate	set 1	set 1							
3	study dialysate	set 1	set 1							
4	study dialysate	set 1	set 1					X	X	
5	study dialysate	set 3	set 2	set 1	set 1	set 1	set 1			
6	study dialysate	set 1	set 1							
7	study dialysate	set 1	set 1							
8	study dialysate	set 1	set 1					X	X	X
9	study dialysate	set 3+A	set 2							
10	standard	set 1	set 1							
11	standard	set 1	set 1							

**Figure 2. Study procedures.** dialysis 1, dialysis 2, dialysis 3: first, second, third dialysis session after the long interdialytic interval of the week respectively; pre: pre-dialysis; post: post-dialysis; blood sampling set 1: lithium heparin gel tube for plasma magnesium measurement; set 2: lithium heparin gel tube for plasma magnesium and potassium measurement; set 3: lithium heparin gel tube for plasma magnesium, potassium, calcium, albumin, phosphate, bicarbonate, CRP, and 2x EDTA tube for hemoglobin and PTH; set A: serum tube for biobanking in participants who provided additional informed consent; set B: 2x EDTA tube for biobanking in participants who provided additional informed consent; ECG: pre-dialysis electrocardiography; Questionnaire: 7-point yes or no questionnaire to ask if a participant experienced the following symptoms in the last week: nausea, vomiting, dizziness, drowsiness, reduced muscle strength, itching and leg cramps, in addition at baseline a question about chronic diarrhea and over-the-counter use of magnesium supplements is included; 3DDD: 3-days dietary diary including 1 dialysis weekday, 1 non-dialysis weekday, 1 non-dialysis weekend day; Holter: 48-hours electrocardiography registration including 1 dialysis session and 1 interdialytic interval; PWV: pulse wave velocity measurement before the mid-week dialysis session.

321x127mm (144 x 144 DPI)



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Abstract & 5
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page
	5b	Name and contact information for the trial sponsor	investigator initiated
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A



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	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
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**Introduction**

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5, 10

**Methods: Participants, interventions, and outcomes**

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-9

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4		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
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7		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
8				
9	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-10
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14	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
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17	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10-11
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21	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5-6
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### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

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27	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
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32	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
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35	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
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Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial	10-11

**Methods: Data collection, management, and analysis**

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7-8, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11

**Methods: Monitoring**

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4	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
5			N/A
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9		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
10			N/A
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12	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
13			8-9
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15	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
16			N/A
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19	<b>Ethics and dissemination</b>		
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21	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
22			5
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24	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
25			N/A
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29	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
30			5-7
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32		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
33			Figure 2
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35	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
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Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
<b>Appendices</b>			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	supp
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7-8, Figure 2

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\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.